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- (54) Recombinant thermostable enzyme for converting maltose into trehalose ...
- (57) Disclosed are a recombinant thermostable enzyme, which converts maltose into trehalose and is stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min, a preparation of the enzyme,

a DNA encoding the enzyme, a recombinant DNA containing the DNA, a transformant, and an enzymatic conversion method of maltose by using the enzyme.

Description

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Background of th Inventi n

Field of the Invention

The present invention relates to a novel recombinant thermostable enzyme which converts maltose into trehalose.

Description of the Prior Art

Trehalose is a disaccharide which consists of 2 glucose molecules linked together with their reducing groups, and, naturally, it is present in bacteria, fungi, algae, insects, etc., in an extremely-small quantity. Having no reducing residue within the molecule, trehalose does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can advantageously sweeten food products without fear of causing unsatisfactory coloration and deterioration. However, trehalose is far from being readily prepared in a desired amount by conventional methods, and, actually, it is not scarcely used for sweetening food products.

Conventional methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other employing a multi-enzymatic system wherein enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154.485/75, is a method which comprises allowing to grow microorganisms such as bacteria and yeasts in a nutrient culture medium, and collecting trehalose from the resultant culture. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and trehalose-phosphorylases to act on maltose, and isolating the formed trehalose from the reaction system. Although the former facilitates the growth of microorganisms without special difficulty, it has a drawback that the resultant culture only contains at most 15 w/w % trehalose, on a dry solid basis (d.s.b.). While the latter enables the separation of trehalose with a relative easiness, but it is theoretically difficult to increase the trehalose yield by allowing enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction per se is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

In view of the foregoing, the present inventors energetically screened enzymes which directly convert maltose into trehalose, and have found that microorganisms belonging to those of the genera *Pimelobacter* and *Pseudomonas*, as disclosed in Japanese Patent Application No.199,971/93, produce an absolutely novel enzyme which forms trehalose when acts on maltose. This means that trehalose can be prepared from maltose as a material which is readily available in quantity and at low cost, and the use of the enzyme would completely overcome all the aforesaid objects.

It was found that all the enzymes from these microorganisms have an optimum temperature of about 20-40°C which seems some how insufficient for trehalose production in their thermostability. It is recognized in this field that the saccharification of starch and amylaceous substances should be generally reacted at a temperature of over 55°C. If the saccharification reaction is effected at a temperature of 55°C or lower, bacterial contamination is enhanced to lower the pH of the reaction mixtures and to inactivate enzymes used, followed by remaining a relatively large amount of substrates intact. If the saccharification reaction is effected by using enzymes with poor thermostability, a great care should be taken for the pH changes, and, once a pH lowering occurs, alkalis should be quickly added to the reaction mixtures to increase the pH.

In view of the foregoing, the present inventors further studied on thermostable enzymes with such activity and have found that enzymes, produced from microorganisms of the genus *Thermus* such as a microorganism of the species *Thermus aquaticus* (ATCC 33923), effectively convert maltose into trehalose without being substantially inactivated even when reacted at a temperature of over 55°C. These enzymes, however, are not sufficient in enzyme producing activity, and this leads to a problem of that an industrial scale production of trehalose inevitably requires a considerably large scale cultivation of such microorganisms.

Recombinant DNA technology has made a remarkable progress in recent years. At present, even an enzyme, whose total amino acid sequence is not revealed, can be readily prepared in a desired amount, if a gene encoding the enzyme was once isolated and the base sequence was decoded, by preparing a recombinant DNA containing a DNA which encodes the enzyme, introducing the recombinant DNA into microorganisms or cells of plants or animals, and culturing the resultant transformants. Under these circumstances, urgently required are to find a gene encoding the above thermostable enzyme and to decode the base sequence.

Summary of the Invention

It is an object of the present invention to provide a recombinant thermostable enzyme which forms trehalose when acts on maltose.

It is a further object of the present invention to provide a DNA which encodes the recombinant enzyme.

It is yet another object of the present invention to provide a replicable recombinant DNA having the DNA.

It is a further object of the present invention to provide a transformant into which the recombinant DNA has been introduced.

It is a further object of the present invention to provide a process for preparing the recombinant enzyme by using the transformant.

It is a further object of the present invention to provide a method for converting maltose into trehalose by the recombinant enzyme.

The first object of the present invention is attained by a recombinant enzyme.

The second object of the present invention is attained by a DNA which encodes the recombinant enzyme.

The third object of the present invention is attained by a replicable recombinant DNA which contains the DNA and a self-replicable vector.

The fourth object of the present invention is attained by a transformant obtained by introducing the replicable recombinant DNA into an appropriate host.

The fifth object of the present invention is attained by culturing the transformant in a nutrient culture medium to form the recombinant enzyme, and collecting the formed recombinant enzyme from the resultant culture.

The sixth object of the present invention is attained by an enzymatic conversion method of maltose which contains a step of allowing the recombinant enzyme to act on maltose to form trehalose.

Brief Description of the Accompanying Drawings

FIG.1 shows the optimum temperature of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.2 shows the optimum pH of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.3 shows the thermal stability of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.4 shows the pH stability of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.5 shows the structure of the recombinant DNA pBTM22 according to the present invention.

FIG.6 shows the structure of the recombinant DNA pBTM23 according to the present invention.

Detailed Description of the Invention

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The recombinant enzyme according to the present invention acts on maltose to form trehalose without being substantially inactivated even when allowed to react at a temperature of over 55°C.

The DNA according to the present invention expresses the production of the present recombinant enzyme when introduced into an appropriate self-replicable vector to obtain a replicable recombinant DNA, then introduced into an appropriate host, which is inherently incapable of forming the recombinant enzyme but readily proliferative, to form a transformant

The recombinant DNA according to the present invention expresses the production of the recombinant enzyme by introducing it into an appropriate host, which is inherently incapable of forming the recombinant enzyme but readily proliferative, to form a transformant, and culturing the transformant in a nutrient culture medium.

The transformant forms a desired amount of the recombinant enzyme when cultured according to the present invention

The enzymatic conversion method according to the present invention converts maltose into a saccharide composition comprising trehalose, glucose and/or maltooligosaccharides.

The present invention was made based on the finding of an absolutely novel thermostable enzyme which converts maltose into trehalose. Such an enzyme can be obtained from cultures of *Thermus aquaticus* (ATCC 33923), and the present inventors isolated the enzyme by using a variety of methods comprising column chromatography as a main technique, and studied on the properties and features, revealing that the reality is a polypeptide having the following physicochemical properties:

(1) Action

Forming trehalose when acts on maltose, and vice versa;

(2) Molecular weight (MW)

About 100,000-110,000 daltons when assayed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE);

(3) Isoelectric point (pl)

About 3.8-4.8 when assayed on isoelectrophoresis;

(4) Optimum temperature

About 65°C when incubated at pH 7.0 for 60 min;

(5) Optimum pH

About 6.0-6.7 when incubated at 60°C for 60 min;

(6) Thermal stability

Stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min; and

(7) pH Stability

Stable up to a pH of 5.5-9.5 even when incubated at 60°C for 60 min.

Experiments for revealing the physicochemical properties of a thermostable enzyme produced from *Thermus aquaticus* (ATCC 33923) are as follows:

Experiment 1

Purification of enzyme

Experiment 1-1

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Production of enzyme

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid culture medium (pH 7.5) containing 0.5 w/v % polypeptone, 0.1 w/v % yeast extract, 0.07 w/v % sodium nitrate, 0.01 w/v % disodium hydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.01 w/v % calcium chloride, and water, and the flasks were autoclaved at 120°C for 20 min to effect sterilization. After cooling the flasks a seed culture of *Thermus aquaticus* (ATCC 33923) was inoculated into each flask, followed by the incubation at 60°C for 24 hours under a rotary-shaking condition of 200 rpm to obtain a seed culture. Twenty L aliquots of a fresh preparation of the same liquid culture medium were put in 30-L jar fermenters, sterilized and cooled to 60°C, followed by inoculating one v/v % of the seed culture into each fermenter, and incubating the resultant at a pH of 6.0-8.0 and 60°C for about 20 hours under aeration-agitation conditions.

Thereafter, the enzymatic activity of the resultant culture was assayed to reveal that it contained about 0.35 units/ml of the enzyme. A portion of the culture was centrifuged, and the supernatant was assayed to reveal that it contained about 0.02 units/ml of the enzyme. While the separated cells were suspended in 50 mM phosphate buffer (pH 7.0) to give the total volume equal to the original volume of the portion, followed by assaying the suspension to reveal that it contained about 0.33 units/ml of the enzyme.

Throughout the specification the enzyme activity is expressed by the value measured on the following assay: Place one ml of 10 mM phosphate buffer (pH 7.0) containing 20 w/v % maltose in a test tube, add one ml of an appropriately diluted enzyme solution to the tube, and incubate the solution in the tube at 60°C for 60 min to effect an enzymatic reaction, followed by a further incubation at 100°C for 10 min to suspend the enzymatic reaction. Thereafter, a portion of the reaction mixture was diluted by 11 times with 50 mM phosphate buffer (pH 7.5), and 0.4 ml of which was placed in a test tube, admixed with 0.1 ml solution containing one unit/ml trehalase, followed by incubating the resultant mixture at 45°C for 120 min and quantifying the glucose content on the glucose oxidase method. As a control, a system using a trehalase solution and an enzyme solution which has been inactivated by heating at 100°C for 10 min is provided and treated similarly as above. The content of the formed trehalose is estimable based on the content of glucose quantified in the above. One unit of the enzyme activity is defined as the amount which forms one µmol trehalose per min under the above conditions.

45 Experiment 1-2

Purification of enzyme

The culture obtained in Experiment 1-1 was centrifuged to separate cells, and about 0.28 kg of the wet cells thus obtained was suspended in 10 mM phosphate buffer (pH 7.0), disrupted in usual manner, and centrifuged to obtain an about 1.8 L of a crude enzyme solution. The solution was admixed with ammonium sulfate to give a saturation of 70 w/v %, salted out by standing at 4°C overnight, and centrifuged to obtain a supernatant. The supernatant was mixed with 10 mM phosphate buffer (pH 7.0), and the mixture solution was dialyzed against a fresh preparation of the same buffer for 24 hours.

The dialyzed inner solution was centrifuged to obtain a supernatant (1,560 ml) which was then applied to a column packed with 530 ml of "DEAE-TOYOPEARL® 650", an ion exchanger commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0), followed by feeding to the column a linear gradient buffer of sodium chloride ranging from 0 M to 0.4 M in 10 mM phosphate buffer (pH 7.0). From the

eluate, fractions with the objective enzyme activity were collected, pooled, dialyzed against 10 mM phosphate buffer (pH 7.0) containing one M ammonium sulfate for 10 hours, and centrifuged to obtain a supermatant. The supernatant was applied to a column packed with 380 ml of "BUTYL-TOYOPEARL® 650", a gel for hydrophobic chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0) containing one M ammonium sulfate, followed by feeding to the column a linear gradient buffer of ammonium sulfate ranging from 1 M to 0 M in 10 mM phosphate buffer (pH 7.0).

Fractions, eluted at 0.2 M ammonium sulfate, with the objective enzyme activity were collected, pooled and dialyzed against 10 mM phosphate buffer (pH 7.0) containing 0.2 M sodium chloride for 16 hours. The dialyzed solution was centrifuged to remove insoluble substances, fed to a column packed with 380 ml of "TOYOPEARL® HW-55S", a gel for gel filtration chromatography commercialized by Tosoh, Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0) containing 0.2 M sodium chloride, followed by feeding to the column with 10 mM phosphate buffer (pH 7.0) containing one M sodium chloride. Fractions with the enzyme activity were collected from the eluate, fed to a column packed with "MONO Q HR5/5" which had been equilibrated with 10 mM phosphate buffer (pH 7.0). The column was fed with a linear gradient buffer of sodium chloride ranging from 0.1 M to 0.35 M in 10 mM phosphate buffer (pH 7.0), followed by collecting fractions with the enzyme activity. The purified enzyme thus obtained had a specific activity of about 135 units/mg protein in a yield of about 330 units per L of the culture.

The purified enzyme was electrophoresed in a 7.5 w/v % polyacrylamide gel to give a single protein band with the enzyme activity, and this meant that it had a considerably-high purity.

Experiment 2

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Physicochemical property of enzyme

Experiment 2-1

Action

To an aqueous solution containing 5 w/w % maltose or trehalose as a substrate was added 2 units/g substrate of the purified enzyme obtained in Experiment 1-2, and the mixture was incubated at 60°C and pH 7.0 for 24 hours. In order to analyze the saccharide composition of the reaction mixture, it was dried *in vacuo*, dissolved in pyridine, and trimethylsilylated in usual manner, and the resultant was subjected to gas chromatography. The equipments and conditions used in this analysis were as follows: "GC-16A" commercialized by Shimadzu Seisakusho, Ltd., Tokyo, Japan, as a gas chromatograph; a stainless steel column, having an inner diameter of 3 mm and a length of 2 m, packed with 2% "SILICONE OV-17/CHROMOSOLB W" commercialized by GL Sciences Inc., Tokyo, Japan, as a column; a hydrogen flame type of ionization as a detector; nitrogen gas as a carrier gas (flow rate of 40 ml/min); and a column oven temperature of 160-320°C at a programmed increasing temperature rate of 7.5°C/min. The saccharide compositions of the reaction mixtures were tabulated in Table 1:

Table 1

Substrate	Saccharide com	position of reacti	on mixture (%)
	Trehalose	Glucose	Maltose
Maltose	70.0	4.4	25.6
Trehalose	76.2	3.1	20.7

As is shown in Table 1, the purified enzyme formed about 70 w/w % trehalose and about 4 w/w % glucose when acted on maltose as a substrate, while it formed about 21 w/w % maltose and about 3 w/w % glucose when acted on trehalose as a substrate. These facts indicate that the purified enzyme has activities of converting maltose into trehalose and of converting trehalose into maltose, as well as of hydrolyzing α l,4 linkage in maltose molecule and α , α -1,1 linkage in trehalose molecule. There has been no report of such an enzyme, and this leads to an estimation of having a novel enzymatic pathway.

Experiment 2-2

Molecular weight

In accordance with the method as reported by U. K. Laemmli in Nature, Vol.227, pp.680-685 (1970), the purified

enzyme was electrophoresed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to give a single protein band at a position corresponding to about 100,000110,000 daltons. The marker proteins used in this experiment were myosin (MW=200,000 daltons), β-galactosidase (MW=116,250 daltons), phosphorylase B (MW=97,400 daltons), serum albumin (MW=66,200 daltons) and ovalbumin (MW=45,000 daltons).

Experiment 2-3

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Isoelectric point

The purified enzyme gave an isoelectric point of about 3.8-4.8 when isoelectrophoresed in 2 w/v % "AMPHOLINE®", a polyacrylamide gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden.

Experiment 2-4

15 Optimum temperature

The optimum temperature of the purified enzyme was about 65°C as shown in FIG.1 when incubated in usual manner in 10 mM phosphate buffer (pH 7.0) for 60 min.

20 Experiment 2-5

Optimum pH

The optimum pH of the purified enzyme was about 6.0-6.7 as shown in FIG.2 when tested in usual manner by incubating it at 60°C for 60 min in 10 mM acetate buffer, phosphate buffer or sodium carbonate/sodium hydrogen carbonate buffer with different pHs.

Experiment 2-6

30 Thermal stability

The purified enzyme was stable up to a temperature of about 80°C as shown in FIG.3 when tested in usual manner by incubating it in 50 mM phosphate buffer (pH 7.0) for 60 min.

35 Experiment 2-7

pH Stability

The purified enzyme was stable up to a pH of about 5.5-9.5 as shown in FIG.4 when experimented in usual manner by incubating it at 60°C for 60 min in 50 mM acetate buffer, phosphate buffer or sodium carbonate/sodium hydrogen carbonate buffer with different pHs.

Experiment 2-8

45 Amino acid sequence containing the N-terminus

The amino acid sequence containing the N-terminus of the purified enzyme was analyzed on "MODEL 470A", a gas-phase protein sequencer commercialized by Perkin-Elmer Corp., Instrument Div., Norwalk, USA, and revealed to have the amino acid sequence containing the N-terminus in SEQ ID NO:1.

SEQ ID NO:1:

Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val 1 5 10 15 Arg Ser Phe Phe

Experiment 2-9

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Partial amino acid sequence

An adequate amount of the purified enzyme prepared in Experiment 1-2 was weighed, dialyzed against 10 mM Tris-HCl buffer (pH 9.0) at 4°C for 18 hours, and admixed with 10 mM Tris-HCl buffer (pH 9.0) to obtain a solution containing about one mg/ml of the enzyme. The solution was incubated at 100°C for 5 min to denature the enzyme, and about one ml of which was placed in a test tube, admixed with 40 µg lysyl endopeptidase, and incubated at 30°C for 44 hours to partially hydrolyze the enzyme. The resultant hydrolysate was applied to "µBONDASPERE C18", a column for reverse-phase high-performance liquid chromatography commercialized by Japan Millipore Ltd., Tokyo, Japan, which had been equilibrated with 0.1 v/v % trifluoroacetate, followed by feeding to the column 0.1 v/v % trifluoroacetate containing acetonitrile at a flow rate of 1.0 ml/min while increasing the concentration of acetonitrile from 0 v/v % to 70 v/v %.

Fractions containing a peptide fragment eluted about 58 min to 60 min after the initiation of the feeding were collected, pooled, dried *in vacuo*, and dissolved in 0.5 ml of 10 mM Tris-HCl buffer (pH 8.0), admixed with 5 µg TPCK treated trypsin, and incubated at 37° C for 16 hours to effect hydrolysis. The enzymatic reaction was suspended by freezing, and the resultant hydrolyzate was fed to a column packed with "µBONDASPERE C18", followed by feeding to the column 0.1 v/v % trifluoroacetate containing aqueous acetonitrile at a flow rate of 1.0 ml/min while increasing the concentration of aqueous acetonitrile from 15 v/v % to 55 v/v %. Fractions, containing a peptide fragment eluted about 42 min after the initiation of the feeding, were collected, pooled, dried *in vacuo*, and dissolved in 0.1 v/v trifluoroacetate containing 50 v/v % aqueous acetonitrile. Similarly as in Experiment 2-8, it was revealed that the peptide fragment contained the amino acid sequence in SEQ ID NO:2.

SEQ ID NO:2:

Since no enzyme with these physicochemical properties has been known, it can be estimated to be a novel substance

The present inventors energetically screened the chromosomal DNA of *Thermus aquaticus* (ATCC 33923) by using an oligonucleotide as a probe which had been chemically synthesized based on the amino acid sequences as revealed in Experiments 2-8 and 2-9, and have obtained a DNA fragment which consisted of about 3,600 base pairs having the base sequence in SEQ ID NO:4. The decoding of the base sequence revealed that a thermostable enzyme from the microorganism consists of 963 amino acids and has the amino acid sequence in SEQ ID NO:3.

SEQ ID NO:3:

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	_			20					25					30	Glu	
			25					40					43		Thr	
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15	Phe				25					90					Ile 95	
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			115					120					123		Asp	
20		120	Lys				135					T-7-0			Glu	
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	Phe				165					1/0					Val 175	
25	_			100					185					エラし	Val	
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			435					440					445	Ala		
	Asn	Gln 450	His	Ala	Lys	Ile	Phe 455	Gly	Arg	Gly	Ser	Leu 460	Thr	Leu	Leu	Pro
5	Val 465	Glu	Asn	Arg	Arg	Val 470	Leu	Ala	Tyr	Leu	Arg 475	Glu	His	Glu	Gly	Glu 480
•	Arg	Val	Leu	Val	Val 485		Asn	Leu	Ser	Arg 490	Tyr	Thr	Gln	Ala	Phe 495	Asp
10	Leu	Pro	Leu	Glu 500		Tyr	Gln	Gly	Leu 505	Val	Pro	Val	Glu	Leu 510	Phe	Ser
	Gln	Gln	Pro 515	Phe	Pro	Pro	Val	Glu 520		Arg	Tyr	Arg	Leu 525	Thr	Leu	Gly
	Pro	His 530		Phe	Ala	Leu	Phe 535	Ala	Leu	Lys	Pro	Val 540	Glu	Ala	Val	Leu
15	His 545	Leu	Pro	Ser	Pro	Asp 550		Ala	Glu	Glu	Pro 555	Ala	Pro	Glu	Glu	Ala 560
	Asp			_	565	His				570				Leu	575	
20	Asp	Thr	Leu	Val 580	His	Glu	Arg	Gly	Arg 585	Glu	Glu	Leu	Leu	Asn 590	Ala	Leu
			595	Leu				600					605	Pro		
		610	Leu				615					620		Pro		
25	625					630					635			Val		640
					645					650				Leu	655	
30	•			660					665					Leu 670		
			675					680					685	Phe		
•	_	690					695					700			0	Pro ·
35	Glu 705	Ala	Val	Asp	Leu	Leu 710		Pro	Gly	Leu	715	Ala	Gly	Glu	GTĀ	720
					725					730				Arg	735	
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			835					840					845	Gly		
55		850					855					860		Leu		
	Glu	Lys	Arg	Gly	Thr	Val	Glu	Glu	Asp	Leu	Ala	Arg	Leu	Ala	Tyr	Asp

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870
               865
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                                                                                                                               890
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               Trp Ala Phe Ala Glu Glu Val Ala Asp His Leu His Ala Ala Phe Leu
                                                                                                                                                                                   910
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                                                      900
               Gln Ala Tyr Arg Ser Ala Leu Pro Glu Glu Ala Leu Glu Glu Ala Gly
                                                                                                                                                                    925
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                Trp Thr Arg His Met Ala Glu Val Ala Ala Glu His Leu His Arg Glu
                930 935 940
Glu Arg Pro Ala Arg Lys Arg Ile His Glu Arg Trp Gln Ala Lys Ala
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                Gly Lys Ala
            SEQ ID NO:4:
15
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             GACTTTGAAA CCTCCAACTG GACCTTTGAC CCCGTGGCCA AGGCCTACTA CTGGCACCGC
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             TTCTACTGGC ACCAGCCCGA CCTCAACTGG GACAGCCCCG AGGTGGAGAA GGCCATCCAC
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            ACCCTAAAGG GCACGCCCAT CGTCTACTAC GGGGACGAGA TCGGCATGGG GGACACCCC 1140
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GGGCGCTACC GCTTGACCCT GGGCCCCAC GGCTTCGCCC TCTTCGCCCT GAAGCCCGTG 1620
GAGGCGGTGC TCCACCTCCC CTCCCCCGAC TGGGCCGAGG AGCCCGCCC CGAGGAGGCC 1680
GACCTGCCC GGGTCCACAT GCCCGGGGG CCGGAGGTCC TCCTGGTGGA CACCCTGGTC 1740
CACGAAAGGG GGCGGAGGA GCCCCTCGCCC AGACCCTGAA GGCAGAAGAC 1800
TGGCTCGCCC TCAAGCCGCA GAAGGTGGCC CTCCTGGACG CCCTCCGCTT CCAGAAGGAC 1860
              CCGCCCTTT ACCTCACCT GCTCCAGCTG GAGAACCACA GGACCCTCCA GGTCTCCTC 1920
CCCCTCCTCT GGTCCCCCA GAGGCGGGAA GGCCCCGGCC TCTTCGCCCG CACCCACGGC 1980
              CAGCCCGGCT ACTTCTACGA GCTCTCCTTG GACCCAGGCT TCTACCGCCT CCTCCTCGCC 2040
              CGCCTTAAGG AGGGGTTTGA GGGGCGGAGC CTCCGGGCCT ACTACCGCGG CCGCCACCCG 2100
              GGTCCCGTGC CCGAGGCCGT GGACCTCCTC CGGCCGGGAC TCGCGGCGGG GGAGGGGGTC 2160
TGGGTCCAGC TCGGCCTCGT CCAAGACGGG GGCCTGGACC GCACGGAGCG GGTCCTCCCC 2220
CGCCTGGACC TCCCCTGGGT TCTCCGGCCC GAAGGGGGCC TCTTCTGGGA GCGGGGCGCC 2280
TCCAGAAAGGG TCCTCGCCCT CACGGGAAGC CTCCCCCCGG GCCGCCCCCA GGACCTCTTC 2340
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              GCCGCCCTGG AGGTCCGGCT CCTGGAAAGC CTTCCCCGCC TCCGGGGGCA CGCCCCGGG 2400
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GTGCGCCTCG CCCCTCCTAG GCCCTGGGCG GTGGAGCGGG GAGGAGGTGG GAGGAGGCCC CTCCACCGGG	TCCTTCCGG CCCTCCTCA GCCGCGGCCT CGGAAAAGCG CCGTGCACCT CCGACCACCT TGGAGGAGGC AGGAAAGGCC	CCGGGCCCTT CGGGGCCTTC GGGCACGGTG CGCCCTCGAG CCACGCCGCC	GGGGAGGTGG CTGGAGCTGG GAGGAGGACC GCCCTGGAGG TTCCTCCAAG	AGGGGGTGGT AGGGGGGGGGT TGGCCCGCCT CGGAGCTTTG CCTACCGCTC	GTACCTCGTG GGCCTACGAC GGCCTTTGCC CGCCCTCCCC GGCGGAGCAC	2580 2640 2700 2760 2820
GGAAAAGCC						

The sequential experimental steps used to reveal the amino acid sequence and the base sequence in SEQ ID NOs:3 and 4 are summarized in the below:

- (1) A thermostable enzyme was isolated from a culture of a donor microorganism, highly purified, and determined for its amino acid sequence containing the N-terminus. The purified enzyme was partially hydrolyzed with protease, and from which a peptide fragment was isolated and determined for its amino acid sequence;
 - (2) Separately, a chromosomal DNA was isolated from a donor microorganism's cell, purified and partially digested with a restriction enzyme to obtain a DNA fragment consisting of about 4,000-8,000 base pairs. The DNA fragment was ligated with a DNA ligase to a plasmid vector, which had been previously cut with a restriction enzyme, to obtain a recombinant DNA;
 - (3) The recombinant DNA was introduced into a microorganism of the species *Escherichia coli* to obtain transformants, and from which an objective transformant containing a DNA encoding the thermostable enzyme was selected by the colony hybridization method using an oligonucleotide, as a probe, which had been chemically synthesized based on the aforesaid partial amino acid sequence; and
 - (4) The recombinant DNA was obtained from the selected transformant and annealed with a primer, followed by allowing a DNA polymerase to act on the resultant to extend the primer, and determining the base sequence of the resultant complementary chain DNA by the dideoxy chain termination method. The comparison of an amino acid sequence, which could be estimated based on the determined base sequence, with the aforesaid amino acid sequence concluded that it encodes the thermostable enzyme.

The following Experiments 3 and 4 concretely illustrate the above items (2) to (4), and the techniques used therein were conventional ones commonly used in this field, for example, those described by J. Sumbruck et al. in "Molecular Cloning A Laboratory Manual", 2nd edition, published by Cold Spring Harbor Laboratory Press (1989).

35 Experiment 3

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Preparation of recombinant DNA containing DNA encoding thermostable enzyme, and transformant

Experiment 3-1

Preparation of chromosomal DNA

A seed culture of *Thermus aquaticus* (ATCC 33923) was inoculated into nutrient broth medium (pH 7.0), and cultured at 60°C for 24 hours with a rotary shaker. The cells were separated from the resultant culture by centrifugation, suspended in TES buffer (pH 8.0), admixed with 0.05 w/v % lysozyme, and incubated at 37°C for 30 min. The resultant was freezed at -80°C for one hour, admixed with TSS buffer (pH 9.0), heated to 60°C, and further admixed with a mixture solution of TES buffer and phenol, and the resultant solution was chilled with ice, followed by centrifugation to obtain a supernatant. To the supernatant was added 2-fold volumes of cold ethanol, and the precipitated crude chromosomal DNA was collected, suspended in SSC buffer (pH 7.1), admixed with 7.5 µg ribonuclease and 125 µg protease, and incubated at 37°C for one hour. Thereafter, a mixture solution of chloroform and isoamyl alcohol was added to the reaction mixture to extract the objective chromosomal DNA, and the extract was admixed with cold ethanol, followed by collecting the formed sediment containing the chromosomal DNA. The resultant purified chromosomal DNA was dissolved in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at -80°C.

Experiment 3-2

Preparation of recombinant DNA pBTM22 and transformant BTM22

About one ml of the purified chromosomal DNA obtained in Example 3-1 was placed in a test tube, admixed with about 10 units of *Sau* 3Al, a restriction enzyme, and enzymatically reacted at 37°C for about 20 min to partially cleave the chromosomal DNA, followed by recovering a DNA fragment consisting of about 4,000-8,000 base pairs by means of sucrose density-gradient ultracentrifugation. One µg of Bluescript II SK(+), a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, was placed in a test tube, subjected to the action of *Bam* HI, a restriction enzyme, to completely digest the plasmid vector, admixed with 10 µg of the DNA fragment and 2 units of T4 DNA ligase, and allowed to stand at 4°C overnight to ligate the DNA fragment to the plasmid vector fragment. To the resultant recombinant DNA was added 30 µl of "Epicurian Coli® XLI-Blue", a competent cell commercialized by Stratagene Cloning Systems, California, USA, Japan, allowed to stand under ice-chilling conditions for 30 min, heated to 42°C, admixed with SOC broth, and incubated at 37°C for one hour to introduce the recombinant DNA into *Escherichia coli*.

The resultant transformant was inoculated into agar plate (pH 7.0) containing 50 μg/ml of 5-bromo-4-chloro-3-in-dolyl-β-galactoside, and cultured at 37°C for 18 hours, followed by placing a nylon film on the agar plate to fix thereon about 6,000 colonies formed on the agar plate. Based on the amino acid sequence of Trp-Tyr-Lys-Asp-Ala-Val as shown in SEQ ID NO:1, the base sequence of probe 1 represented by the base sequence of 5'-TGGTAYAARGAYGCNGT-3' was chemically synthesized, labelled with ³²P, and hybridized with the colonies of transformants fixed on the nylon film, followed by selecting 5 transformants which had strongly hybridized with the probe 1.

The objective recombinant DNA was selected in usual manner from the 5 transformants, and, in accordance with the method described by E. M. Southern in *Journal of Molecular Biology*, Vol.98, pp.503-517 (1975), the-recombinant DNA was hybridized with probe 2 represented by the base sequence of 5'-AAYATGTGGCCNGARGA-3', which had been chemically synthesized based on the amino acid sequence in SEQ ID NO:2, i.e. Asn-Met-Trp-Pro-Glu-Glu, and labelled with ³²P, followed by selecting a recombinant DNA which had strongly hybridized with the probe 2. The recombinant DNA and the transformant thus selected were respectively named "pBTM22" and "BTM22".

The transformant BTM22 was inoculated into L-broth (pH 7.0) containing 100 µg/ml ampicillin, and cultured at 37°C for 24 hours by a rotary shaker. After completion of the culture, the resultant cells were centrifugally collected from the culture, and treated with conventional alkaline method to extract a recombinant DNA from the cells. The extract was in usual manner purified and analyzed and revealing that the recombinant DNA pBTM22 consists of about 10,300 base pairs. As is shown in FIG.5, a fragment containing a DNA, which consists of about 2,900 base pairs and encodes the thermostable enzyme, is located in the downstream near the digested site of *Hind* III, a restriction enzyme.

Experiment 3-3

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Production of recombinant enzyme by transformant BTM22

In 500-ml flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % glucose. 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % dipotassium hydrogen phosphate, 0.06 w/v % sodium dihydrogen phosphate, 0.05 w/v % magnesium sulfate heptahydrate, 0.5 w/v % calcium carbonate and water, and each flasks was sterilized by heating at 115°C for 30 min, cooled, admixed with 50 µg/ml ampicillin, and inoculated with the transformant BTM22 obtained in Experiment 3-2, followed by culturing the transformant at 37°C for 24 hours by a rotary shaker. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supernatant thus obtained was assayed for the enzyme activity and revealing that one L of the culture contained about 800 units of a recombinant enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Thermus aquaticus* (ATCC 33923) was inoculated in a fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Thermus aquaticus* (ATCC 33923), it was cultured and treated similarly as above except that the cultivation temperature was set to 65°C. Assaying the activity of the resultant, one L culture of *Thermus aquaticus* contained about 350 units of the enzyme, and the yield was significantly lower than that of transformant BTM22. *Escherichia coli* XLI-Blue used as a host did not form the thermostable enzyme.

Thereafter, the enzyme produced by the transformant BTM22 was purified similarly as in Experiments 1 and 2, and examined for its physicochemical properties and features. As a result, it was revealed that it has substantially the same physicochemical properties as the thermostable enzyme of *Thermus aquaticus* (ATCC 33923), i.e. it has a molecular weight of about 100,000-110,000 daltons on SDS-PAGE and an isoelectric point of about 3.8-4.8 on isoelectrophoresis, and is not substantially inactivated even when incubated at 80°C for 60 min in water (pH 7.0). The results indicate that the present thermostable enzyme can be prepared by recombinant DNA technology, and the yield can be significantly increased thereby.

Experiment 4

Preparation of complementary chain DNA and determination for its base sequence and amino acid sequence

Two μg of the recombinant DNA pBTM22 in Experiment 3-2 was placed in a test tube, admixed with 2 M aqueous sodium hydroxide solution to effect degeneration, and admixed with an adequate amount of cold ethanol, followed by collecting the formed sediment containing a template DNA and drying the sediment *in vacuo*. To the template DNA were added 50 pmole/ml of a chemically synthesized primer represented by the base sequence of 5'-GTAAAACGACG-GCCAGT-3', 10 μ l of 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM magnesium chloride and 20 mM sodium chloride, and the mixture was incubated at 65°C for 2 min to effect annealing and admixed with 2 μ l of an aqueous solution containing dATP, dGTP and dTTP in respective amounts of 7.5 μ M, 0.5 μ l of [α -32P]dCTP (2 mCi/ml), one μ l of 0.1 M dithiothreitol, and 2 μ l of 1.5 units/ml T7 DNA polymerase, followed by incubating the resultant mixture at 25°C for 5 min to extend the primer from the 5'-terminus to the 3'-terminus. Thus, a complementary chain DNA was formed.

The reaction product containing the complementary chain DNA was divided into four equal parts, to each of which 2.5 µl of 50 mM aqueous sodium chloride solution containing 80 µM dNTP and 8 µM ddATP, ddCTP, ddGTP or ddTTP was added, and the resultant mixture was incubated at 37°C for 5 min, followed by suspending the reaction by the addition of 4 µl of 98 v/v % aqueous formamide solution containing 20 mM EDTA, 0.05 w/v % bromophenol blue, and 0.05 w/v % xylene cyanol. The reaction mixture was heated with a boiling-water bath for 3 min, and a small portion of which was placed on a 6 w/v % polyacrylamide gel, and electrophoresed by energizing it with a constant voltage of about 2,000 volts to separate DNA fragments, followed by fixing the gel in usual manner, drying it and subjecting the resultant to autoradiography.

Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of about 3,600 base pairs in SEQ ID NO:5. An amino acid sequence estimable from the base sequence was as shown in parallel in SEQ ID NO:5, and it was compared with the amino acid sequence containing the N-terminus or the partial amino acid sequences in SEQ ID NOs:1 and 2 and revealing that the amino acid sequence in SEQ ID NO:1 corresponded to that positioning from 1 to 20 in SEQ ID NO:5, and the amino acid sequence in SEQ ID NO:2 corresponded to that positioning from 236 to 250 in SEQ ID NO:5. These results indicate that the present recombinant enzyme has the amino acid sequence in SEQ ID NO:3, and the amino acid sequence of the DNA derived from *Thermus aquaticus* (ATCC 33923) is encoded by the base sequence in SEQ ID NO:4.

SEQ ID NO:5:

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GCCCTCCT CCCCAAC TGCTCGACG GGAGGTGC CTTCCCTCT ACCCCGGG CGGGGGCG AGGCGAC GAGGGCTCC TGGGGGCC AAGCCCAAGG GGGTGAC	G CCCCTCTTGC T GCGGGTGGAG A GAAGGCCCGG T GGGCCTCGAG	GCCGTGGGCC GACAAGGGCT GCCTGCCTCG GCCCTCCCCG	TCGCCCTGGC AGGCCTGGCT GCAAGAGGGT	CCTGCACTAC TAAGGCGGTG CCTGGAGCTC	60 120 180 240 300 360
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5	GTG	GAC	CCC	CTC	TGG	TAC Tyr	AAG	GAC	GCG	GTG Val	ATC	TAC	CAG	CTC	CAC His	GTC	588
	1 CGC	TCC	TTC Phe	TTT Phe	5 GAC Asp	GCC Ala	AAC Asn	AAC Asn	GAC Asp	GGC Gly	TAC Tyr	GGG Gly	GAC Asp	TTT Phe	15 GAG Glu	GGC Gly	636
10	_			20		CCC			25					30			684
	Leu	Arg	Arg 35	Lys	Leu	Pro	Tyr	Leu 40	Glu	Glu	Leu	Gly	Val 45	Asn	Thr	Leu	
	TGG	CTC	ATG	CCC	TTC	TTC	CAG	TCC	CCC	TTG	AGG	GAC	GAC	GGG	TAC	GAT	732
15	-	50				Phe	55					60					760
	ATC	TCC	GAC	TAC	TAC	CAG	ATC	CTC	CCC	GTC	CAC	GGG	ACC	CTG	GAG	GAC	780
	65		_			Gln 70					75					80	828
	TTC	ACC	GTG	GAC	GAG	GCC Ala	CAC	GGC	CGG	Clar	ATG	LAG	Ual	ATC	TIA	GAG	020
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	CCG	GAG	AAG	TAC	AAG	GGG	GTC	CGG	GTC	ATC	TTC	AAG	GAC	TTT	GAA	ACC	972
		130		_		Gly	135				•	140					
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	145		_			Asp 150		•			155					160	1060
	TTC	TAC	TGG	CAC	CAG	CCC	GAC	CTC	AAC	TGG	GAC	AGC	CCC	GAG	GTG	GAG	1068
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	AAG	GCC	ATC	CAC	CAG	GTC Val	ATG	TTC	TTC	1.GG	A 3 m	ACD	LAN	GGG	Ual	ACD	1110
	_			180		GCC			185					190			1164
40	Gly	Phe	Arg	Leu	Asp	Ala	Ile	Pro 200	Tyr	Leu	Tyr	Glu	Arg 205	Glu	Gly	Thr	1101
	TCC	ጥርር		AAC	CTC	CCC	GAG		ATT	GAG	GCG	GTG		CGC	CTG	AGG	1212
	Ser	Cys 210	Glu	Asn	Leu	Pro	Glu 215	Thr	Ile	Glu	Ala	Val 220	ГЛE	Arg	Leu	Arg	
	AAG		CTG	GAG	GAG	CGC		GGC	CCC	GGG	AAG	ATC	CTC	CTC	GCC	GAG	1260
45	Lys 225	Ala	Leu	Glu	Glu	Arg 230	Tyr	Gly	Pro	Gly	Lys 235	Ile	Leu	Leu	Ala	Glu 2 4 0	
	GCC	AAC	ATG	TGG	CCG	GAG	GAG	ACC	CTC	CCC	TAC	TTC	GGG	GAC	GGG	GAC	1308
	Ala	Asn	Met	Trp	Pro 245	Glu	Glu	Thr	Leu	Pro 250	Tyr	Phe	Gly	Asp	Gly 255	Asp	
50	GGG	GTC	CAC	ATG	GCC	TAC	AAC	TTC	CCC	CTG	ATG	CCC	CGG	ATC	TTC	ATG	1356
50	_			260		Tyr			265					270			
	GCC	CTA	AGG	CGG	GAG	GAC	CGG	GGT	CCC	ATT	GAA	ACC	ATG	CTC	AAG	GAG	1404
	Ala	Leu	Arg	Arg	Glu	Asp	Arg	Gly	Pro	Ile	Glu	Thr	Met	Leu	Lys	GLU	

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	GCG	GAG	GGG	ATC	CCC	GAA	ACC	GCC	CAG	TGG	81a	Leu	Phe	Len	CGC	Asn	1402
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10					775					330							1506
	ATC	CGC	CGC	CGC	CTC	ATG	CCC	CTC	CTC	GGG	GGC	GAC	CGC	AGG	CGG	TAC	1596
	Ile	Arg	Arg	Arg	Leu	Met	Pro	Leu	Leu	GIA	GIA	ASP	Arg	350	Arg	IYI	
																	1644
	GAG	CTC	CTC	ACC	GCC	CTC	CTC	CTC	Thr	LOU	Lare	GGC	Thr	Pro	ATC	Val	
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	TAC	TAC	Cle	ACD	GAG	Tle	Glv	Met	Gly	Asp	Asn	Pro	Phe	Leu	Gly	Asp	
	CGG		GGT	GTC	AGG	ACC	CCC	ATG	CAG	TGG	TCC	CAA	GAC	CGC	ATC	GTC	1740
20	Arg	Asn	Glv	Val	Arg	Thr	Pro	Met	Gln	Trp	Ser	Gln	yeb	Arg	Ile		
						200					393						1788
		TTC	TCC	CGC	GCC	CCC	TAC	CAC	GCC	CTC	TTC	CTT	DEC	Pro	GTG	Ser	1700
	Ala	Phe	Ser	Arg	Ala	Pro	Tyr	His	ATB	Leu	Pne	Leu	FIU	FIU	Val 415	UCI	
05					405		636	mmc	CTC	410	GTG	GAG	GCC	CAG	CGG	GAA	1836
25	GAG	GGG	CCC	TAC	AGC	TAC	UAC	Pho	Val	Asn	Val	Glu	Ala	Gln	Arg	Glu	
	N N C	CCC	CAC	mcc	CTC	CTG	AGC	TTC	AAC	CGC	CGC	TTC	CTC	GCC	CTG	AGG	1884
	AAC	Phe	His	Ser	Leu	Leu	Ser	Phe	Asn	Arg	Arg	Phe	Deu	Ala	Leu	Arg	
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00	AAC	CAG	CAC	GCC	AAG	ATC	TTC	GGC	CGG	GGG	AGC	CTC	ACC	UTT	CTC	Pro	1932
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	CTC	ccc	TTG	GAG	GCC	TAC	CAA	GGC	CTC	GTC	CCC	GTG	GAG	CTC	TTC	Ser	2070
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5	Ala	Gln	Thr	Leu	Lys	Glu	Lys	Ser	Trp	Leu	ATA	Leu	605	PIO	GIII	пуз	0410
	GTG	GCC	ama	CTG	GAC	GCC	CTC	CGC	TTC	CAG	AAG	GAC	CCG	CCC	CTT	TAC	2412
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			CTG	CTC	CAG	CTG	GAG	AAC	CAC	AGG	ACC	CTC	CAG	GTC	TCC	CTC	2460
10	Leu	Thr	Leu	Leu	Gln	Leu	Glu	Asn	ніз	Arg	635	peu	Gin	AGI	561	640	
	625	CTC	CTC	TGG	TCC	000	CAG	AGG	CGG	GAA	GGC	CCC	GGC	CTC	TTC	GCC	2508
	Pro	Leu	Leu	Trp	Ser	Pro	Gln	Arg	arg	650	GIY	FIU	Gry	Dea	655		
15	CGC	ACC	CAC	GGC	~ ~ ~	CCC	GGC	TAC	TTC	TAC	GAG	CTC	TCC	TTG	GAC	CCA	2556
	Arg	Thr	His	Gly	Gln	Pro	Gly	Tyr	665	TYP	Giu	пеп	361	670	nap		2424
	GGC	TTC	TAC		CTC	CTC	CTC	GCC	CGC	CTT	AAG	GAG	GGG	TTT	GAG	GGG	2604
	Gly	Phe	Tyr	Arg	Leu	Leu	Leu	ALA	Arg	reu	гÃ2	G1u	685	1116	0.1.4	CII	0650
20	CGG	AGC		CGG	GCC	TAC	TAC	CGC	GGC	CGC	CAC	CCG	GGT	CCC	GTG	CCC	2652
	Arg	Ser	Leu	Arg	Ala	TYI	TYT	Arg	GTA	Arg	nrs	700	013				2700
	GAG	GCC	GTG	GAC	CTC	CTC	CGG	CCG	GGA	CTC	GCG	GCG	CIGG	CAG	Gly	Val	2700
	Glu	Ala	Val	Asp	Leu	Leu	Arg	Pro	Gly	Leu	715	nru	GIY	GIU	Gry	720	
25		GTC															2748
	TGG	GTC Val	CAG	CTC	GGC	CTC	Unl	CAA	Acn	Glv	Glv	Leu	Asp	Ara	Thr	Glu	
	000	GTC	CTC	CCC		ÓMO	GAC	CTC	ccc	TCC	CTT	CTC	CGG	CCC	GAA	GGG	2796
30	Arg	Val	Leu	Pro	Arg	Leu	Asp	Leu	7/5	тгр	Vai	Leu	Ary	750		0-1	
	GGC	CTC	ттС	maa	GAG	CGG	GGC	GCC	TCC	AGΛ	AGG	GTC	CTE	GCC	CTC	ACG	2844
	Gly	Leu	Phe	Trp	Glu	Arg	GIY	760	SET	ary	ALG	144	765				2892
	GGA	AGC	CTC	ccc	CCG	GGC	CGC	CCC	CAG	GAC	CTC	TTC	GCC	GCC	CTG	Clu	2092
35	Gly	Ser	Leu	Pro	Pro	Gly	Arg	Pro	GIN	Asp	Leu	780	YTO	, nru	LCu		2940
	GTC	CGG	CTC	CTG	GAA	AGC	CTT	CCC	CGC	CTC	CGG	GGG	CAC	33 e	Bro	GGG Glv	23.0
	Val	Arg	Leu	Leu	Glu	Ser	Leu	Pro	Arg	reu	795	GLY	nis	, nio		800	2988
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		Pro			205					010							3036
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50		0-6					95	5				וסס	,			Ala	3180
	GA.			GGC	ACG	GTG	GAC	GAC	GAC	CTG	GCC	CGC	CTG	, GCC	TAU	- Aen	5250
	G1: 865	ı Lys	Arg	g Gly	Thr	7 Va3	. GI	ı Glı	ı Ası) Le	875	. WI	j rei	3 ATS	а туг	880	

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Trp	Ala	Phe	Ala	GAG Glu	Glu	Val	Ala	ASP 905	HIS	Leu	ure	WIG	910	TTC Phe	Dea	3276
Gln	Ala	Tyr	CGC Arg	Ser	Ala	Leu	920	GAG Glu	GIU	WIG	Deu	925	014	GCG Ala	0-1	3324
Trp	Thr	Arg	His	Met	Ala	Glu	GTG Val	АТа	ATB	GIU	940	Dea		CGG Arg	010	3372
Glu	930 AGG Arg	CCC Pro	GCC Ala	CGC Arg	Lys	CCC	ATC Ile	CAC His	GAG Glu	CGC Arg 955	115	CAG Gln	GCC Ala	AAG Lys	GCC Ala 960	3420
		GCC Ala			950					,,,						3429
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As is described above, the present thermostable enzyme capable of converting maltose into trehalose and *vice versa* which was found as a result of the present inventors' long-term research, and, unlike conventional enzymes, the enzyme has a specific physicochemical properties. The present invention aims to prepare a recombinant enzyme by means of recombinant DNA technology. Referring the following examples, the process for preparing such a recombinant enzyme, its preparation and uses will be described in detail.

The recombinant enzyme as referred to in the present invention includes those in general which are prepared by recombinant DNA technology and capable of converting maltose into trehalose and *vice versa*. Usually the present recombinant DNA has a revealed amino acid sequence, e.g. the amino acid sequence in SEQ ID NO:3 or a homologous amino acid to it. Variants containing amino acid sequences, which are homologous to the amino acid sequence in SEQ ID NO:3, can be prepared by replacing one or more amino acids in SEQ ID NO:3 with other amino acids without alternating the inherent activity of the enzyme. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, as well as on ingredients and components of nutrient culture media used for culturing transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have the enzymatic activity inherent to the enzyme encoded by the DNA but defect one or more amino acids located in neamess to the N-and/or the C-termini of the amino acid sequence in SEQ ID NO:3, or have one or more amino acids newly added to the N-terminus by the modification of intracellular enzymes of hosts after the DNA expression. Such variants can be included in the present recombinant enzyme as long as they have the desired properties.

The recombinant enzyme according to the present invention can be obtained from cultures of transformants containing the specific DNA. Transformants usable in the present invention can be obtained by introducing into appropriate hosts the base sequence in SEQ ID NO:4, homologous base sequences to it, or complementary base sequences to these base sequences. One or more bases in the above mentioned base sequences may be replaced with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence for which they code. Needless to say, one or more bases in the base sequence, which encodes the enzyme or their variants, can be readily replaced with other bases to allow the DNA to actually express the enzyme production in hosts.

Any DNA derived from natural resources and those artificially synthesized can be used in the present invention as long as they have the aforementioned base sequences. The natural resources of the DNA according to the present invention are, for example, microorganisms of the genus *Thermus aquaticus* (ATCC 33923) from which a gene, containing the DNA used in the present invention, can be obtained. These microorganisms can be inoculated into nutrient culture media and cultured for about 1-3 days under aerobic conditions, and the resultant cells were collected from cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or β-glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used in combination with the cell-wall lysis enzyme, and, in the case of treating the cells with ultrasonication, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or treated with the freezing and thawing method. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment used in general in this field. To artificially synthesize the DNA according to the present invention, it can be chemically synthesized by using the base sequence in SEQ ID NO:3, or can be obtained in plasmid form by inserting a DNA, which encodes the amino acid sequence in SEQ ID NO:4, into an appro-

priate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the recombinant DNA from the cells.

Such a recombinant DNA, for example, in the form of a recombinant DNA, is usually introduced into hosts. Generally the recombinant DNA contains the aforesaid DNA and a self-replicable vector and can be prepared by conventional method with a relative easiness when the material DNA is in hand. Examples of such a vector are plasmid vectors such as pBR322, pUC18, Bluescript II SK(+), pKK223-3, pUB110, pTZ4, pC194, pHV14, TRp7, TEp7, pBS7, etc.; and phage vectors such as λgt-λC, λgt-λB, p11, φ1, φ105, etc. Among these plasmid- and phage-vectors, pBR322, pUC18, Bluescript II SK(+), pKK223-3, λgt-λC and λgt-λB are satisfactorily used in case that the present DNA should be expressed in *Escherichia coli*, while pUB110, pTZ4, pC194, p11, φ1 and φ105 are satisfactorily used to express the DNA in microorganisms of the genus *Bacillus*. The plasmid vectors pHV14, TRp7, TEp7 and pBS7 are suitably used when the recombinant DNA is allowed to grow in 2 or more types of hosts.

The methods used to insert the present DNA into such vectors in the present invention may be conventional ones generally used in this field. A gene containing the present DNA and a self-replicable vector are first digested by a restriction enzyme and/or ultrasonic disintegrator, then the resultant DNA fragments and vector fragments are ligated. To ligate DNA fragments and vectors, they may be annealed if necessary, then subjected to the action of a DNA ligase in vivo or in vitro. The recombinant DNA thus obtained is replicable without substantial limitation by introducing it into an appropriate host, and culturing the resultant transformant.

The recombinant DNA according to the present invention can be introduced into appropriate host microorganisms including *Escherichia coli* and those of the genus *Bacillus* as well as actinomyces and yeasts. In the case of using *Escherichia coli* as a host, it can be cultured in the presence of the recombinant DNA and calcium ion, while in the case of using the microorganisms of the genus *Bacillus* the competent cell method and the colony hybridization method can be employed. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing either maltose or trehalose and selecting transformants which form trehalose or maltose.

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The transformants thus obtained extracellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid media in general supplemented with carbon sources, nitrogen sources and/or minerals, and, if necessary, further supplemented with a small amount of amino acids and/or vitamins can be used as the nutrient culture media. Examples of the carbon sources are saccharides such as starch, starch hydrolysate, glucose, fructose and sucrose. Examples of the nitrogen sources are organic- and inorganig-substances containing nitrogen such as ammonia, ammonium salts, urea, nitrate, peptone, yeast extract, defatted soy been, corn steep liquor and beef extract. Cultures containing the objective enzyme can be obtained by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 20-50°C and a pH of 2-9 for about 1-6 days under aerobic conditions by aeration-agitation, etc. Such cultures can be used intact as a crude enzyme preparation, and, usually, cells in the cultures can be disrupted with ultrasonic disintegrator and/or cell-wall lysis enzymes prior to use, followed by separating the enzyme from intact cells and cell debris by filtration and/or centrifugation, and purifying the enzyme. The methods used for purifying the enzyme in the invention include conventional ones in general. From cultures intact cells and cell debris are removed and subjected to one or more methods such as concentration, salting out, dialysis, separately sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectrophoresis.

As is described above, the present recombinant thermostable enzyme exerts a distinct activity of forming trehalose or maltose from maltose or trehalose respectively even when allowed to act at a temperature of over 55°C, and such an activity has not been found in conventional enzymes. Trehalose has a mild and high-quality sweetness and it has a great advantage of being capable of sweetening food products without fear of causing unsatisfactorily coloration and deterioration because it has no reducing residue within the molecule. By using these properties of the present recombinant thermostable enzyme, maltose, which could not have been used in some field due to its reducibility, can be converted into useful trehalose with a satisfactory handleability and substantial no reducibility.

Explaining now the present enzymatic conversion method in more detail, the wording "maltose" as referred to in the present invention usually means a saccharide composition containing maltose, and any material or method can be used in the present invention as long as trehalose is formed when the present recombinant thermostable enzyme acts thereon or formed thereby. To effectively produce trehalose in an industrial scale, saccharide compositions with a relatively-high maltose content, i.e., usually, about 70 w/w % or more, preferably, about 80 w/w % or more, can be arbitrarily used. Such saccharide compositions can be prepared by conventional methods generally used in this field, for example, those as disclosed in Japanese Patent Publication Nos. 11,437/81 and 17,078/81 wherein β-amylase is allowed to act on gelatinized- or liquefied-starch and separating the formed maltose by separation-sedimentation method or dialysis method, or those as disclosed in Japanese Patent Publication Nos. 13,089/72 and 3,938/79 wherein β-amylase is allowed to act on gelatinized- or liquefied-starch together with a starch debranching enzyme such as isoamylase or pullulanase.

In the enzymatic conversion method according to the present invention, an effective amount of the present recom-

binant thermostable enzyme is allowed to coexist in an aqueous medium containing maltose, followed by keeping the resultant mixture at a prescribed temperature and pH to enzymatically react until the desired amount of trehalose is formed. Although the enzymatic reaction proceeds even at a relatively-low concentration of about 0.1 w/w %, d.s.b., the concentration may be set to about 2 w/w % or more, d.s.b., preferably, about 5-50 w/w %, d.s.b., to proceed the enzymatic conversion method in an industrial scale. The reaction temperature and pH are set within the range which effectively forms maltose without inactivating the recombinant enzyme, i.e. a temperature of over 55°C, preferably, about 56-63°C, and a pH of about 5-10, preferably, about 6-7. The amount of the recombinant enzyme and the reaction time are appropriately set depending on the conditions of the enzymatic reaction. The present enzymatic conversion method effectively converts maltose into trehalose, and the conversion rate reaches up to about 50% or more in some cases.

The reaction mixtures obtainable by the present enzymatic conversion method can be used intact, and, usually, they may be purified prior to use. For example, the reaction mixtures are filtered and centrifuged to remove insoluble substances, and the resultant solutions are decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated into syrupy products. Depending on use, the syrupy products can be dried *in vacuo* and spray-dried into solid products. To obtain products substantially consisting of trehalose, the syrupy products are subjected to one or more methods of chromatographies using ion exchangers, activated charcoals or silica gels, fermentation using yeasts, and removal by decomposing reducing saccharides with alkalis. To treat a relatively-large amount of reaction mixtures, ion-exchange chromatographies such as fixed bed-, moving bed-, and pseudo-moving bed-methods as disclosed in Japanese Patent Laid-Open Nos.23,799/83 and 72,598/83 are arbitrarily used in the invention, and these enable the effective and large production of high-trehalose content products which have been difficult to obtain in large quantities.

The trehalose and saccharide compositions containing trehalose thus obtained can be used in a variety of products which should be avoided from the reducibility of saccharide sweeteners, and therefore, they can be arbitrarily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stability, filler, adjuvant or excipient.

The following examples explain the preparation of the recombinant thermostable enzyme and the enzymatic conversion method of maltose according to the present invention:

Example A-1

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Preparation of recombinant enzyme

To 500-ml Erlenmeyer flasks were added 100 ml aliquots of a nutrient culture medium consisting of 2.0 w/v % glucose, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % dipotassium hydrogen phosphate, 0.06 w/v % sodium dihydrogen phosphate, 0.05 w/v % magnesium sulfate heptahydrate, 0.5 w/v % calcium carbonate and water, and each flask was sterilized by heating at 115°C for 30 min, cooled, admixed with 50 μg/ml ampicillin, and inoculated with the transformant BTM22 obtained in Experiment 1-2, followed by the incubation at 37°C for 24 hours under rotatory-shaking conditions to obtain a seed culture. To 30-L jar fermenters were added 18 L aliquots of a fresh preparation of the same nutrient culture medium, sterilized similarly as above, admixed with 50 μg/ml ampicillin, and inoculated with 1 v/v % of the seed culture, followed by the incubation at 37°C and a pH of 6-8 for 24 hours under aeration-agitation conditions. The resultant cultures were pooled, treated with ultrasonication to disrupt cells, centrifuged to remove insoluble substances, followed by assaying the enzymatic activity of the resultant supernatant. As a result, one L of the culture contained about 800 units of the recombinant enzyme. The assay of the supernatant conducted by the method in Experiment 1-1 revealed that in this culture was obtained an about 5 ml aqueous solution containing about 152 units/ml of a recombinant enzyme with a specific activity of about 135 units/mg protein.

Example A-2

Preparation of recombinant thermostable enzyme

50 Example A-2(a)

Preparation of transformant BTM23

Recombinant DNA pBTM22, obtained by the method in Example 3-2, was cleaved with *Hind* III, a restriction enzyme, to obtain a DNA fragment consisting of about 8,100 base pairs which contain the base sequence positioning from 107 to 2,889 in SEQ ID NO:4.

Eight oligonucleotides containing base sequences represented by 5'-AGCTTGAATTCTTTTTTAATAAAATCAGGAGGAAAAACCATGGA CC-3', 5'-CCCTCTGGTACAAGGACGCGGTGATCTACCAGCTCCAC-3',5'-GTCCGCT CCT-

TCTTTGACGCCAACACGACGGCTACGG-3', 5'-GGACTTTGAGGGCCTGAGG CGGA-3', 5'-AGCTTCCGCCTCAGGCCCTCAAAGTCCCCGTAGCCGTCGTTGTTG-3', 5'-GCGTCAAAGAAGAAGGAGCGGACGTGGAGCTGGTAGATCACC-3', 5'-GCGTCCTTG TACCAGAGGGGGTCCATGGTTTTTCCTCC-3', and 5'-TGATTTTATTAAAAAAGAA TTCA-3 were mixed in adequate amounts, and the mixture was successively incubated at 100°C 65°C, 37°C and 20°C for 20 min, respectively, to anneal the oligonucleotides. A first recombinant DNA, which contains the base sequence in SEQ ID NO:6 and a base sequence consisting of the bases of positions 1-2,889 in SEQ ID NO:3 wherein the guanines (G) located in the positions 1-963 were replaced with adenines (A), was obtained by adding the above DNA fragment to a double stranded DNA of 141 base pairs having 5' cohesive end of 4 bases at each terminus, which consists of the base sequence in SEQ ID NO:6 and the bases of positions 1-110 in SEQ ID NO:4 wherein the guanine (G) located in the position 1 in SEQ ID NO:4 was replaced with adenine (A) without alternating the amino acid sequence consisting of those of positions 1-36 in SEQ ID NO:3, and allowing the mixture to stand at 4°C overnight in the presence of T4 DNA ligase to anneal the contents.

SEQ ID NO:6:

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AGCTTGAATT CTTTTTTAAT AAAATCAGGA GGAAAAACC

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Recombinant DNA pBTM22 obtained by the method in Experiment 3-2 was cleaved with *Bam* HI, a restriction enzyme, to obtain a DNA fragment consisting of about 2,400 base pairs which contains the base sequence positioning from 1,008 to 2,889 in SEQ ID NO:4 which was then ligated with "M13tv19 RF DNA", a phage vector commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, which had been cleaved with *Bam* HI to obtain a second recombinant DNA.

An oligonucleotide containing a base sequence represented by 5'-CGGTAGCCCTGCAGCCCCGGG-3' corresponding to the base sequence positioning at 3,438 to 3,458 in SEQ ID NO:5, where "thymine (T)", the base positioning at 3,448 in SEQ ID NO:5 was replaced with "guanine (G)", was in usual manner chemically synthesized. By using the synthesized oligonucleotide and "MUTAN-G", a site-specific mutation system commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, a third recombinant DNA, which contained the base sequence positioning from 1,008 to 2,889 bases in SEQ ID NO:4 where "thymine (T)", i.e. the base positioning at 3,448 in SEQ ID NO:5, was replaced with "guanine (G)" without alternating the amino acid sequence positioning from 337 to 963 bases in SEQ ID NO:5 which was contained in the second recombinant DNA, was obtained. The procedure of site-specific mutation followed the manual affixed to the "MUTAN-G".

A DNA fragment, consisting of about 1,390 base pairs containing the base sequence positioning at 1 to 1,358 bases in SEQ ID NO:4 where "guanine (G)", i.e. the first base in SEQ ID NO:4, was replaced with "adenine (A)", obtained by cleaving with restriction enzymes *Eco* RI and *BgI* II, and a DNA fragment consisting of abut 1,550 base pairs containing the base sequence positioning at 1,359 to 2,889 in SEQ ID NO:4 obtained by cleaving the third recombinant DNA with restriction enzymes *BgI* II and *Pst* I, were ligated to "pKK223-3", a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, with T4 DNA ligase to obtain the recombinant DNA pBTM23 containing the base sequence in SEQ ID NO:4.

The recombinant DNA pBTM23 thus obtained was introduced into *Escherichia coli* LE 392 (ATCC 33572) which had been previously prepared into a competent cell according to the method as described by J. Sambrook in "*Molecular Cloning, A Laboratory Manual*", 2nd edition, pp.1.74-1.81 (1989), published by Cold Spring Harbor Laboratory Press, New York, USA, to obtain the present transformant BTM23 containing the DNA coding for the present enzyme. The transformant was cultured by the method in Experiment 3-2, and the proliferated cells were collected from the resultant culture, and lysed to extract the recombinant DNA which was then purified and analyzed, revealing that the recombinant DNA pBTM23 in FIG.6 consisted of about 7,500 base pairs and had a DNA fragment containing 2,889 base pairs which was ligated to the downstream of *Nco* I, a restriction enzyme.

Example A-2(b)

Preparation of recombinant thermostable enzyme using transformant

The transformant BTM23 was cultured similarly as in Example A-1 except that a liquid culture medium (pH 7.0) consisting of one w/v % maltose, 3 w/v % polypeptone, one w/v % "MEAST P1G", a product of Asahi Breweries, Ltd., Tokyo, Japan, 0.1 w/v % sodium dihydrogen phosphate dihydrate, 200 µg/ml ampicillin sodium and water was used. To the resultant culture were added lysozyme from albumen, commercialized by Seikagaku-Kogyo Co., Ltd., Tokyo, Japan, and "TRITON X-100", a surfactant to give respective concentrations of 0.1 mg/ml; and 1 mg/ml, and the resultant was incubated at 37°C for 16 hours while stirring to extract a recombinant thermostable enzyme from the cells. The suspension was heated at 60°C for one hour to inactivate concomitant enzymes from Escherichia coli, followed by centrifuging the mixture to remove impurities, and assaying the enzyme activity in the supernatant, revealing that one L culture contained about 120.000 units of the recombinant thermostable enzyme. The supernatant was purified by the m thod

in Experiment 1 to obtain an about 177 ml aqueous solution containing about 1,400 units/ml of the recombinant thermostable enzyme with a specific activity of about 135 units/mg protein.

The properties and features of the purified enzyme were studied by the method Experiment 2, revealing that it has a molecular weight of 100,000-110,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and an isoelectric point of about 3.8-4.8 on isoelectrophoresis, and it is not inactivated even when incubated at 80°C for 60 min in an aqueous solution (pH 7.0). These physicochemical properties are substantially the same of those of *Thermus aquaticus* (ATCC 33923) as a donor microorganism.

Example B-1

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Preparation of trehalose syrup by recombinant enzyme

Potato starch powder was suspended in water to give a concentration of 10 w/w %, and the suspension was adjusted to pH 5.5, admixed with 2 units/g starch of "SPITASE HS", an α-amylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and heated at 95°C to effect gelatinization and liquefaction. Thereafter, the resultant liquefied solution was autoclaved at 120°C for 20 min to inactivate the remaining enzyme, promptly cooled to 50°C, adjusted to pH 5.0, admixed with 500 units/g starch of an isoamylase specimen commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and 20 units/g starch of a β-amylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and subjected to an enzymatic reaction at 50°C for 24 hours to obtain a saccharide solution containing about 92 w/w % maltose, d.s.b. The saccharide solution was heated at 100°C for 20 min to inactivate the remaining enzyme, cooled to 60 C, adjusted to pH 6.5, admixed with one unit/g starch of the recombinant enzyme obtained in Example A-1, and subjected to an enzymatic reaction for 96 hours. The reaction mixture was heated at 100°C for 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and deionized with an ion-exchange resin, and concentrated to obtain a 70 w/w % syrup in a yield of about 95% to the material starch, d.s.b.

The product contains about 68 w/w % trehalose, d.s.b, and has a relatively-low reducibility because of its DE (dextrose equivalent) 18.4, as well as having a mild sweetness, moderate viscosity and, moisture-retaining ability, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, stabilizer, filler, adjuvant or excipient.

Example B-2

Preparation of trehalose powder by recombinant DNA

The reaction mixture obtained in Example B-1 was adjusted to pH 5.0, admixed with 10 units/g starch of "GLU-COZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and subjected to an enzymatic reaction at 50°C for 24 hours. The reaction mixture thus obtained was heated to inactivate the remaining enzyme, and, in usual manner, decolored, desalted, purified and subjected to ion-exchange column chromatography using "XT-1016 (polymerization degree of 4%)", a cation exchange resin in Na*form commercialized by Tokyo Organic Chemical Industries., Ltd., Tokyo, Japan, to increase the trehalose content. More particularly, the ion-exchange resin, previously suspended in water, was packed in 4 jacketed-stainless steel columns with an inner column diameter of 5.4 cm, and the columns were cascaded in series to give a total column length of 20 m. About 5 v/v % of the reaction mixture was fed to the columns while the inner column temperature was keeping at 60°C, and fractionated by feeding to the columns with 60°C hot water at an SV (space velocity) 0.15, followed by collecting high-trehalose content fractions. The fractions were pooled, and, in usual manner, concentrated, dried in *vacuo*, and pulverized to obtain a trehalose powder in a yield of about 50% to the material, d.s.b.

The product, which contains about 97 w/w % trehalose, d.s.b, and has a relatively-low reducing power and a mild sweetness, can be arbitrarily incorporated into a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetner, taste-improving agent, stabilizer, filler, adjuvant or excipient.

Example B-3

Preparation of crystalline trehalose powder by recombinant enzyme

A high-trehalose content fraction, obtained by the method in Example B-2, was in usual manner decolored with an activated charcoal, desalted with an ion-exchanger, and concentrated into an about 70 w/w % solution. The concentrate was placed in a crystallizer and gradually cooled while stirring to obtain a massecuite with a crystallization percentage of about 45%. The massecuite was sprayed at a pressure of about 150 kg/cm² from a nozzle equipped at the top of a

drying tower while about 85°C hot air was blowing downward from the top of the drying tower, about 45°C hot air was blowing through under a wire-netting conveyer, which was equipped in the basement of the drying tower, to a crystalline powder collected on the conveyer, and the powder was gradually conveying out from the drying tower. Thereafter, the crystalline powder was transferred to an aging tower and aged for 10 hours in the stream of hot air to complete the crystallization and drying. Thus, a hydrous crystalline trehalose powder was obtained in a yield of about 90% to the material, d.s.b.

The product is substantially free from hygroscopicity and readily handleable, and it can be arbitrarily used in a variety compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stability, filler, adjuvant or excipient.

Example B-4

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Preparation of anhydrous crystalline trehalose powder by recombinant enzyme

A high-trehalose content fraction, obtained by the method in Example B-2, was purified similarly as in Example B-3, and the resultant solution was transferred to a vessel and boiled under a reduced pressure to obtain a syrup with a moisture content of about 3.0 w/w %. The syrup was placed in a crystallizer, admixed with about 1.0 w/w % anhydrous crystalline trehalose as a seed crystal, crystallized at 120°C while stirring, and transferred to a plain aluminum vessel, followed by aging the contents at 100°C for 6 hours to form a block. The block thus obtained was pulverized with a cutter, dried by fluidized bed drying to obtain an anhydrous crystalline trehalose powder with a moisture content of about 0.3 w/w % in a yield of about 85% to the material, d.s.b.

The product with a strong dehydrating activity can be arbitrarily used as a desiccant for food products, cosmetics and pharmaceuticals, as well as their materials and intermediates, and also used as a white powdery sweetener with a mild sweetness in food products, cosmetics and pharmaceuticals.

Example B-5

Preparation of trehalose powder by recombinant enzyme

"MALTOSE HHH", a high-purity maltose commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was dissolved in water to give a concentration of 40 w/w %, heated to 57°C, adjusted to pH 6.5, mixed with 2 units/g maltose, d.s.b., of a recombinant thermostable enzyme obtained by the method in Example A-2, followed by the enzymatic reaction for 48 hours. The reaction mixture was heated at 100°C for 10 min to inactivate the remaining enzyme, cooled, filtered, decolored with an activated charcoal in usual manner, desalted and purified with an ion-exchange resin, dried *in vacuo*, and pulverized to obtain a powdery product containing about 73 w/w % trehalose, d.s.b., in a yield of about 90% to the material maltose, d.s.b.

Although the product has a DE (dextrose equivalent) of 19 which is about 30% of that of maltose, it has the same viscosity as that of maltose, as well as having a mild sweetness and an adequate moisture-retaining ability. Thus, the product can be arbitrarily used as a sweetener, quality-improving agent, stabilizer, filler, adjuvant and excipient in a variety of compositions such as food products, cosmetics and pharmaceuticals.

As is described above, the present invention is based on the finding of a novel thermostable enzyme which forms trehalose or maltose when acts on maltose or trehalose. The present invention aims to explore a way to produce such an enzyme in an industrial scale and in a considerably-high yield by recombinant DNA technology. The enzymatic conversion method using the present recombinant thermostable enzyme converts maltose into a saccharide composition containing trehalose, glucose and/or maltose in a considerably-high yield. Trehalose has a mild and high-quality sweetness, and does not have a reducing residue within the molecule, and because of these it can readily sweeten food products in general without fear of causing unsatisfactory coloration and deterioration. The recombinant enzyme with a revealed amino acid sequence can be used with a greater safety for the preparation of trehalose which is premised to be used in food products.

Therefore, the present invention is a useful invention which exerts the aforesaid significant action and effect as well as giving a great contribution to this field.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirit and scope of the invention.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT:

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- (ii) TITLE OF INVENTION:RECOMBINANT THERMOSTABLE ENZYME FOR CONVERTING MALTOSE INTO TREHALOSE
 - (iii) NUMBER OF SEQUENCES:6
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 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear
 - (ii) MOLECULE TYPE:peptide
 - (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:
- Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val
 1 5 10 15
 Arg Ser Phe Phe
 20
 - (3) INFORMATION FOR SEQ ID NO:2:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH:15 amino acids
 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

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(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

Ile Leu Leu Ala Glu Ala Asn Met Trp Pro Glu Glu Thr Leu Pro 1 5 10 15

(4) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH:963 amino acids
 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:peptide
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

	(XI) S	EQUE	VCE DE	301111	11014.0		110.0.									
	Met 1	Asp	Pro	Leu	Trp 5	Tyr	Lys	Asp	Ala	Val 10	Ile	Tyr	Gln	Leu	His 15	Val
20	Ārg	Ser		20					25					30		
		Arg	35	-				40					45			
25	•	Leu 50					55					60				
	65	Ser				70					75					80
30		Thr			85					90					95	
		Val		100					105					110		
	_	Lys	115					120					125			-
35 ,		Glu 130	_	_			135					140				
	145	Asn	_			150					155					160
40		Tyr			165					170					175	
		Ala		180					185					190		
	_	Phe	195					200					205			
45		Cys 210					215					220				
	225	Ala				230					235					240
50		Asn			245					250					255	
	_	Val		260					265		•			270		
		Leu	275					280					285			
55		Glu 290	-				295					300				
	His	Asp	Glu	Leu	Thr	Leu	Glu	Lys	Val	Thr	Glu	GLu	GIU	Arg	GLU	rne

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310
                                             315
    305
    Met Tyr Glu Ala Tyr Ala Pro Asp Pro Lys Phe Arg Ile Asn Leu Gly
                                         330
                     325
    Ile Arg Arg Arg Leu Met Pro Leu Leu Gly Gly Asp Arg Arg Tyr
                                     345
                                                         350
                 340
    Glu Leu Leu Thr Ala Leu Leu Leu Thr Leu Lys Gly Thr Pro Ile Val
                                 360
     Tyr Tyr Gly Asp Glu Ile Gly Met Gly Asp Asn Pro Phe Leu Gly Asp
                             375
                                                 380
         370
10
     Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val
                         390
                                             395
     Ala Phe Ser Arg Ala Pro Tyr His Ala Leu Phe Leu Pro Pro Val Ser
                                                             415
                                         410
                     405
     Glu Gly Pro Tyr Ser Tyr His Phe Val Asn Val Glu Ala Gln Arg Glu
                                    425
                 420
     Asn Pro His Ser Leu Leu Ser Phe Asn Arg Arg Phe Leu Ala Leu Arg
                                 440
                                                     445
            435
     Asn Gln His Ala Lys Ile Phe Gly Arg Gly Ser Leu Thr Leu Leu Pro
                                                 460
                             455
     Val Glu Asn Arg Arg Val Leu Ala Tyr Leu Arg Glu His Glu Gly Glu
20
                         470
                                             475
     Arg Val Leu Val Val Ala Asn Leu Ser Arg Tyr Thr Gln Ala Phe Asp
                                         490
                     485
     Leu Pro Leu Glu Ala Tyr Gln Gly Leu Val Pro Val Glu Leu Phe Ser
                                                        510
25
                                     505
                 500
     Gln Gln Pro Phe Pro Pro Val Glu Gly Arg Tyr Arg Leu Thr Leu Gly
                                                     525
                                 520
     Pro His Gly Phe Ala Leu Phe Ala Leu Lys Pro Val Glu Ala Val Leu
                                                 540
                             535
     His Leu Pro Ser Pro Asp Trp Ala Glu Glu Pro Ala Pro Glu Glu Ala
30
                                             555
                         550
     Asp Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu Val
                                         570
                     565
     Asp Thr Leu Val His Glu Arg Gly Arg Glu Glu Leu Leu Asn Ala Leu
                                     585
                                                         590
                 580
     Ala Gln Thr Leu Lys Glu Lys Ser Trp Leu Ala Leu Lys Pro Gln Lys
                                                     605
                                 600
     Val Ala Leu Leu Asp Ala Leu Arg Phe Gln Lys Asp Pro Pro Leu Tyr
                                                 620
                             615
     Leu Thr Leu Leu Gln Leu Glu Asn His Arg Thr Leu Gln Val Ser Leu
40
                                             635
                         630
     Pro Leu Leu Trp Ser Pro Gln Arg Arg Glu Gly Pro Gly Leu Phe Ala
                     645
                                         650
     Arg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro
                 660
                                     665
45
     Gly Phe Tyr Arg Leu Leu Leu Ala Arg Leu Lys Glu Gly Phe Glu Gly
                                                     685
                                 680
     Arg Ser Leu Arg Ala Tyr Tyr Arg Gly Arg His Pro Gly Pro Val Pro
                             695
                                                 700
     Glu Ala Val Asp Leu Leu Arg Pro Gly Leu Ala Ala Gly Glu Gly Val
                                             715
                         710
     Trp Val Gln Leu Gly Leu Val Gln Asp Gly Gly Leu Asp Arg Thr Glu
                                         730
                     725
     Arg Val Leu Pro Arg Leu Asp Leu Pro Trp Val Leu Arg Pro Glu Gly
                                     745
     Gly Leu Phe Trp Glu Arg Gly Ala Ser Arg Arg Val Leu Ala Leu Thr
```

			755		•			760					765	_		
	_	770	Leu				775					780	Ala			
5		Arg				790					795		His			800
•	Thr				BOS					8TO			Glu		972	
10	_			82A	Val				825				Ala	030		
			025	Val				840					Arg 845			
15		0 = 0	Leu				855					800	Ala			
,,	065	Lys				ደፖበ					8/3		Leu			000
	Val				225					טפט			Glu		0 2 3	
20	_			$\alpha \alpha \alpha$	Glu				905				Ala	910		
			015	Arg				920					943			Gly
25	_	020	Arg				935					940	Leu			
	Glu 945	Arg	Pro	Ala	Arg	Lys 950	Arg	Ile	His	Glu	Arg 955	Trp	Gln	Alą	Lys	Ala 960
	-	Lys	Ala													

(5) INFORMATION FOR SEQ ID NO:4:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:2889 base pairs (B) TYPE:nucleic acid (D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

	(,,,,						
40			GGACGCGGTG	ATCTACCAGC-	TECACGTCCG	CTCCTTCTTT	60
	GTGGACCCCC	.0.00	• • • • • • •	GAGGGCCTGA	GGCGGAAGCT	TCCCTACCTG	120
	GACGCCAACA	ACGACGGCTA	CGGGGACTTT			CTTGAGGGAC	180
	GAGGAGCTCG	GGGTCAACAC	CCTCTGGCTC	ATGCCCTTCT	TCCAGTCCCC	CCTGGAGGAC	240
	GACGGGTACG	ATATCTCCGA	CTACTACCAG	ATCCTCCCCG	TCCACGGGAC		300
	TTCACCGTGG	ACGAGGCCCA	CGGCCGGGGG	ATGAAGGTGA	TCATTGAGCT	CGTCCTGAAC	
45	CACACCTCCA	TTGACCACCC	TTGGTTCCAG	GAGGCGAGGA	AGCCGAATAG	CCCCATGCGG	360
	GACTGGTACG	TGTGGAGCGA	CACCCGGAG	AAGTACAAGG	GGGTCCGGGT	CATCTTCAAG	420
		CCTCCAACTG	GACCTTTGAC	CCCGTGGCCA	AGGCCTACTA	CTGGCACCGC	480
	GACTTTGAAA	••••	CCTCAACTGG	GACAGCCCCG	AGGTGGAGAA	GGCCATCCAC	540
	TTCTACTGGC	ACCAGCCCGA		GTGGACGGCT	TCCGCCTGGA	CGCCATCCCC	600
	CAGGTCATGT	TCTTCTGGGC	CGACCTGGGG		CCGAGACCAT	TGAGGCGGTG	660
50	TACCTCTACG	AGCGGGAGGG	GACCTCCTGC			CCTCGCCGAG	720
	AAGCGCCTGA	GGAAGGCCCT	GGAGGAGCGC		GGAAGATCCT	GGTCCACATG	780
	GCCAACATGT	GGCCGGAGGA	GACCCTCCCC		ACGGGGACGG		840
	GCCTACAACT	TCCCCCTGAT	GCCCCGGATC	TTCATGGCCC	TAAGGCGGGA	GGACCGGGGT	
	CCCATTGAAA	CCATGCTCAA	GGAGGCGGAG	GGGATCCCCG	AAACCGCCCA	GTGGGCCCTC	900
	TTCCTCCGCA	ACCACGACGA	GCTCACCCTG	GAGAAGGTCA	CGGAGGAGGA	GCGGGAGTTC	960
55		CCTACGCCCC	CGACCCCAAG		ACCTGGGGAT	CCGCCGCCGC	1020
35	ATGTACGAGG		CGACCGCAGG		TCCTCACCGC	CCTCCTCCTC	1080
	CTCATGCCCC	TCCTCGGGGG		•••	TCGGCATGGG	GGACAACCCC	1140
	ACCCTAAAGG	GCACGCCCAT	CGTCTACTAC				1200
	TTCCTCGGGG	ACCGGAACGG	TGTCAGGACC	CCCATGCAGT	GGTCCCAAGA	0000110010	

	CCCTTCTCCC	CCCCCCTA	CCACGCCCTC	TTCCTTCCCC	CCGTGAGCGA	GGGGCCCTAC	1260
•	AGCTACCACT	TCGTCAACGT	GGAGGCCCAG	CGGGAAAACC	CCCACTCCCT	CCTGAGCTTC	1320
	AACCGCCGCT	TCCTCGCCCT					1380
5	ACCCTTCTCC	CCGTGGAGAA	CCGCCGCGTC	CTCGCCTACC	TGAGGGAGCA	CGAGGGGGAG	1440
•	CGGGTCCTGG	TGGTGGCCAA	CCTCTCCCGC	TACACCCAGG	CCTTTGACCT	CCCCTTGGAG	1500
	GCCTACCAAG	GCCTCGTCCC			AACCCTTCCC	CCCGGTGGAG	1560
	GGGCGCTACC		GGGCCCCCAC	GGCTTCGCCC	TCTTCGCCCT	GAAGCCCGTG	1620
	GAGGCGCTACC	TCCACCTCCC	CTCCCCGAC	TGGGCCGAGG	AGCCCGCCCC	CGAGGAGGCC	1680
	GACCTGCCCC			CCGGAGGTCC	TCCTGGTGGA		1740
10	CACGAAAGGG	GGCGGGAGGA			AGACCCTGAA	GGAGAAGAGC	1800
	TGGCTCGCCC			CTCCTGGACG	CCCTCCGCTT	CCAGAAGGAC	1860
	CCGCCCCTTT	ACCTCACCCT		GAGAACCACA	GGACCCTCCA	GGTCTCCCTC	1920
	CCCCTCCTCT	GGTCCCCCCA	GAGGCGGGAA	GGCCCCGGCC	TCTTCGCCCG	CACCCACGGC	1980
	CAGCCCGGCT	ACTTCTACGA		GACCCAGGCT	TCTACCGCCT	CCTCCTCGCC	2040
15	CGCCTTAAGG	AGGGGTTTGA		CTCCGGGCCT	ACTACCGCGG	CCGCCACCCG	2100
	GGTCCCGTGC	CCGAGGCCGT	GGACCTCCTC	CGGCCGGGAC	TCGCGGCGGG		2160
	TGGGTCCAGC	TCGGCCTCGT	CCAAGACGGG	GGCCTGGACC	GCACGGAGCG	GGTCCTCCCC	
	CGCCTGGACC	TCCCCTGGGT	TCTCCGGCCC	GAAGGGGGCC	TCTTCTGGGA	•	2280
	TCCAGAAGGG	TCCTCGCCCT	CACGGGAAGC	CTCCCCCGG	GCCGCCCCCA		2340
20	GCCGCCCTGG	AGGTCCGGCT	CCTGGAAAGC	CTTCCCCGCC	TCCGGGGGCA	CGCCCCGGG	2400
20	ACCCCAGGCC	TCCTTCCCGG	GGCCCTGCAC	GAGACCGAAG	CCCTGGTCCG	CCTCCTCGGG	2460
	GTGCGCCTCG	CCCTCCTCCA	CCGGGCCCTT	000000100	AGGGGGTGGT	GGGGGGCCAC	2520
	CCCCTCCTAG	GCCGCGGCCT	CGGGGCCTTC	CTGGAGCTGG	AGGGGGAGGT	GTACCTCGTG	2580
	GCCCTGGGCG	CGGAAAAGCG	GGGCACGGTG	GAGGAGGACC	TGGCCCGCCT	GGCCTACGAC	2640
	GTGGAGCGGG	CCGTGCACCT	CGCCCTCGAG	GCCCTGGAGG	CGGAGCTTTG	GGCCTTTGCC	2700
25	GAGGAGGTGG	CCGACCACCT	CCACGCCGCC	TTCCTCCAAG	CCTACCGCTC	CGCCCTCCCC	2760
	GAGGAGGCCC	TGGAGGAGGC	GGGCTGGACG	CGGCACATGG	CCGAGGTGGC	GGCGGAGCAC	2820
	CTCCACCGGG	AGGAAAGGCC	CGCCCGCAAG	CGCATCCACG	AGCGCTGGCA	GGCCAAGGCC	2880
•	GGAAAAGCC						2889

(6) INFORMATION FOR SEQ ID NO:5:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:3600 base pairs
- (B) TYPE:nucleic acid
- (C) STRANDEDNESS:DOUBLE
- (D) TOPOLOGY:linear
- 40 (ii) MOLECULE TYPE:genomic DNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Thermus aquaticus
 - (B) INDIVIDUAL ISOLATE: ATCC 33923
 - (ix) FEATURE:

(A)NAME/KEY:5'UTR
(B)LOCATION:1..540

50 (C)IDENTIFICATION METHOD:E
(A)NAME/KEY:mat peptide
(B)LOCATION:541..3429
(C)IDENTIFICATION METHOD:S
(A)NAME/KEY:3'UTR

55 (B)LOCATION:3430..3600
(C)IDENTIFICATION METHOD:E

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

	GCC	CCTC	CCT	CCCC	CAACO	eg go	CCT	rccc(cttg(TGG GCC	GGGG GTGG	GCC	GCAC GTGA	AGCC'	rg G	AGGA/ CGGGC	AGGGG CCAGG	60 12 0
5	CGGG GAGG AAGC	GGGCT	CGG A	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	GAGG GCCT GACA	A GA T GG A GG	AGGC GCCA GCCA	CCGG CGAC AGCG	GCC GCC GCC GCC	TGCC CTCC CTCA	TCG CCG AGGC	AGGC GCAA TCCI CCGC	CTGG GAGG CGGA	GT T GT C CG C	'AAGG CTGG CACC GCCT	ACTAC CGGTG AGCTC CGGAC TAAGG	180 240 300 360 420 480
10	GGCC	CGGG		CACC	CTGG	CCT	ACTI	GCAZ	ACC	TACC	TCC	GACC	CACT	'AG C	CTTT	AGGCC	540
	CMC	CAC	יטט ר	CTC	TGG	TAC	AAG	GAC	GCG	GTG	ATC	TAC	CAG	CTC	CAC	GTC	588
	Met	Asp	Pro	Leu	Trp	Tyr	Lys	Asp	Ala	Val 10	Ile	Tyr	GIn	Leu	15	vaı	
	CCC	TCC	TTC	TTT	GAC	GCC	AAC	AAC	GAC	GGC	TAC	GGG	GAC	TTT	GAG	GGC	636
15	Arg	Ser	Phe	Phe	Asp	Ala	Asn	Asn	Asp	Gly	Tyr	Gly	Asp	Pne	Glu	Gly	
	_			20					25					30			601
	CTG	AGG	CGG	AAG	CTT	CCC	TAC	CTG	GAG	GAG	CTC	GGG	GTC	AAC	ACC	Tou	684
		_	25					40					45		Thr		732
00	TGG	CTC	ATG	CCC	TTC	TTC	CAG	TCC	CCC	TTG	AGG	GAC	ACD	GGG	TAC	ACD	732
20	Trp		Met	Pro	Phe	Phe	55 55	Ser	PTO	Leu	Arg	60	veh	GLY	Tyr	nsp	
	N TO C	50 TCC	GAC	TAC	TAC	CAG	ATC	CTC	CCC	GTC	CAC	GGG	ACC	CTG	GAG	GAC	780
	TIO	Ser	Asn	TVT	Tvr	Gln	Ile	Leu	Pro	Val	His	Gly	Thr	Leu	Glu	Asp	
	65					70					75					80	
25	ጥጥር	ACC	GTG	GAC	GAG	GCC	CAC	GGC	CGG	GGG	ATG	AAG	GTG	ATC	ATT	GAG	828
	Phe	Thr	Val	Asp	Glu	Ala	His	Gly	Arg	Gly	Met	Lys	Val	Ile	Ile 95	Glu	
	000		CEC	3 3 C	85 CAC	NCC.	Tr.C.C	አ ምጥ	GAC	90 CAC	ССТ	TĠG	TTC	CAG	GAG	GCG	876
	CTC	Ual	Leu	AAC	His	Thr	Ser	Ile	Asp	His	Pro	Trp	Phe	Gln	Glu	Ala	
				100					105					TIO			
30	AGG	AAG	CCG	ייעע	AGC	CCC	ATG	CGG	GAC	TGG	TAC	GTG	TGG	AGC	GAC	ACC	924
	Arg	Lys	Pro	Asn	Ser	Pro	Met	Arg	Asp	Trp	Tyr	Val	Trp 125	Ser	Asp	Thr	
			115			000	CMC	120	CTC	איזירי	מיזיני	AAG		ттт	GAA	ACC	972
	CCG	GAG	AAG	TAC	LVC	Clar	Val	Ara	Val	Tle	Phe	Lvs	Asp	Phe	Glu	Thr	
95		120				•	135					140					
35	TCC	220	TGG	ACC	TTT	GAC	CCC	GTG	GCC	AAG	GCC	TAC	TAC	TGG	CAC	CGC	1020
	Ser	Asn	Trp	Thr	Phe	Asp	Pro	Val	Ala	Lys	Ala	Tyr	Tyr	Trp	His	Arg	
	1 4 5					150					155					100	1068
	TTC	TAC	TGG	CAC	CAG	CCC	GAC	CTC	AAC	TGG	GAC	AGC	CCC	GAG	GTG	Clu	1000
40	Phe	Tyr	Trp	His	Gln 165	Pro	Asp	Leu	Asn	17p	Asp	Ser	Pro	GIU	Val 175	Giu	
	N N C	ccc	ልጥር	CAC	CAG	GTC	ATG	TTC	TTC	TGG	GCC	GAC	CTG	GGG	GTG	GAC	1116
	Lvs	Ala	Ile	His	Gln	Val	Met	Phe	Phe	Trp	Ala	Asp	Leu	Gly	Val	Asp	
	_			180					185					TAO			1164
	GGC	TTC	CGC	CTG	GAC	GCC	ATC	CCC	TAC	CTC	TAC	GAG	CGG	GAG	GGG	ACC	1164
45	Gly	Phe		Leu	Asp	Ala	Ile	Pro	Tyr	Leu	Tyr	GIU	205	GIU	Gly	TILL	
			195				~~~	200							CTG	AGG	1212
	TCC	TGC	GAG	AAC	CTC	CCC	Clas	Thr	A11	GAU	λla	Val	Lvs	Arg	CTG Leu	Ara	
		210					215					220					
	AAG	GCC	CTG	GAG	GAG	CGC	TAC	GGC	CCC	GGG	AAG	ATC	CTC	CTC	GCC	GAG	1260
50	Lys	Ala	Leu	Glu	Glu	Arg	Tyr	Gly	Pro	Gly	Lys	ITE	Leu	Leu	Ala	GIU	
	225					230					235					240	1308
	GCC	AAC	ATG	TGG	CCG	GAG	GAG	ACC	CTC	CCC	TAC	TTC	GGG	GAC	GGG) and	1300
	Ala	Asn	Met	Trp			Glu	Tur	Leu	250	ıyı	rne	GTĀ	лзр	Gly 255	rap	
		CmC	010	a mo	245	mac	ת א א	ጥጥር	CCC	23U	ልጥር	CCC	CGG	ATC	TTC		1356
55	فافائ	GTC	CAC	ATG	GUU	TAC	AAC	110		C10	NI G		233				

	Gly	Val	His	Met 260	Ala	Tyr	Asn	Phe	Pro 265	Leu	Met	Pro	Arg	Ile 270	Phe	Met	
	~~~	Cm N	3 CC	CCC	GNG	CAC	CGG	CCT	CCC	ATT	GAA	ACC	ATG		AAG	GAG	1404
5	Ala	Leu	Arg	Arg	Glu	Asp	Arg	Gly 280	Pro	Ile	Glu	Thr	Met 285	Leu	Lys	Glu	
	GCG Ala	GAG Glu	CCC	ATC Ile	CCC Pro	GAA Glu	Thr	GCC Ala	CAG Gln	TGG Trp	GCC Ala	Leu	TTC Phe	CTC Leu	CGC Arg	AAC Asn	1452
		290					295		CITIC	100	CNC	300	CAC	ccc	CAC	መጥር	1500
10	His	GAC Asp	GAG Glu	CTC	Thr	Leu	GAG	Lys	Val	Thr	Glu 315	Glu	Glu	Arg	GAG Glu	Phe 320	1300
	305	m » C	CAC	CCC	ሞአር	310	CCC	GAC	CCC	AAG	TTC	CGC	ATC	AAC	CTG		1548
	Mot	TAC	GAG	Ala	Tyr	Ala	Pro	Asp	Pro	Lvs	Phe	Arg	Ile	Asn	Leu	Gly	
		-			325					330					335		
15	ATC	CGC	CGC	CGC	CTC	ATG	CCC	CTC	CTC	GGG	GGC	GAC	CGC	AGG	CGG	TAC	1596
	Ile	Arg	Arg	Arg	Leu	Met	Pro	Leu	Leu 345	Gly	Gly	yab	Arg	AFG 350	Arg	TYT	1014
	GAG	CTC	CTC	ACC	GCC	CTC	CTC	CTC	ACC	CTA	AAG	GGC	ACG	CCC	ATC	GTC	1644
20			255					360					305		Ile		1692
20	TAC	TAC	GGG	GAC	GAG	ATC	GGC	ATG	GGG	GAC.	AAC	CCC	Pho	LOU	GGG	Acn	1092
•	-	270					375					380			Gly		1740
	CGG	AAC	GGT	GTC	AGG	ACC	CCC	Mot	CAG	TGG	Ser	Gln	Asn	Ara	ATC Ile	Val	1,40
25		Asn	GLA	VAI	Arg	390	PIU	Met	GIII	ПЪ	395	02		9		400	
25	385	ттС	TCC	CGC	GCC	CCC	TAC	CAC	GCC	CTC	TTC	CTT	CCC	CCC	GTG	AGC	1788
	Ala	Phe	Ser	Arg	Ala 405	Pro	Tyr	His	Ala	Leu 410	Phe	Leu	Pro	PFO	415	ser	
	GAG	GGG	CCC	TAC	AGC	TAC	CAC	TTC	GTC	AAC	GTG	GAG	GCC	CAG	CGG	GAA	1836
30	Glu	Gly	Pro	Tyr	Ser	Tyr	His	Phe	Val 425	Asn	Val	Glu	YIa	430	Arg	GIU	1004
	AAC	CCC	CAC	TCC	CTC	CTG	AGC	TTC	AAC	CGC	CGC	TTC	CTC	GCC	CTG	AGG	1884
			435					440					445		Leu		1932
	AAC	CAG	CAC	GCC	AAG	ATC	TTC	GGC	CGG	GGG	AGC	CTC	Thr	Len	CTC	Pro	1952
35		450					455					400			Leu		1980
	GTG	GAG	AAC	CGG	CGC	GTC	LOV	212	TAC	Len	AUG	Glu	His	Glu	Gly	Glu	
	165					470					475					480	2028
40	CGG	GTC	CTG	GTG	GTG	GCC	AAC	CTC	TCC	CGC	TAC	ACC	CAG	A Top	TTT	ASD	2028
					485					490					Phe 495		2076
	CTC	ccc	TTG	GAG	GCC	TAC	CAA	GGC	CTC	GTC	Pro	Un1	GAG	Leu	TTC Phe	Ser	2070
				500					505					210			2124
45	CAG	CAA	CCC	TTC	CCC	CCG	GTG	GAG	Cle	DGC Ara	TAC	Ara	TAN	Thr	CTG	Glv	2124
			515					520					<b>525</b>		Leu GTG		2172
	ccc	CAC	GGC	TTC	GCC	CTC	TTC	Ala	CTG	Lare	Pro	Val	Glu	Ala	GTG Val	Leu	
50		530					535					540					2220
	CAC	CTC	CCC	TCC	CCC	GAC	TGG	GCC	GAG	GAG	CCC	GCC	Pro	GAG G1.	GAG Glu	Ala	2220
	H1s 545		Pro	Ser	PTO	Asp 550	тrр	YTS	GLU	GIU	555	WIG	FIO	GIU	Glu	560	

	GAC	CTG	ccc	CGG	GTC	CAC	ATG	CCC	GGG	GGG	CCG	GAG	GTC	CTC	CTG	GTG	2268
•	_	Leu			565					570					575		
5	GAC	ACC	CTG	GTC	CAC	GAA	AGG	GGG	CGG	GAG	GAG	CTC	CTA	AAC	GCC	CTC	2316
3	- Asp			SRO					585					590			
	GCC	CAG	ACC	CTG	AAG	GAG	AAG	AGC	TGG	CTC	GCC	CTC	AAG	CCG	CAG	AAG	2364
	Ala	Gln	Thr	Leu	Lys	Glu	Lys	Ser 600	Trp	Leu	Ala	Leu	Lys 605	PTO	GIN	rĀa	
10	GTG	GCC	CTC	CTG	GAC	GCC	CTC	CGC	TTC	CAG	AAG	GAC	CCG	CCC	CTT	TAC	2412
	Val	Ala.	Leu	Leu	Asp	Ala	Leu 615	Arg	Phe	Gln	Lys	Asp 620	Pro	PTO	гÃ2	TYL	
	CTC	ACC.	CTG	CTC	CAG	CTG	GAG	AAC	CAC	AGG	ACC	CTC	CAG	GTC	TCC	CTC	2460
	Leu	Thr	Leu	Leu	${\tt Gln}$	Leu	Glu	Asn	His	Arg	Thr	Leu	Gln	Val	Ser	Leu	
15	625					630					635	000	000	CIIIC	mmc	640 CCC	2508
	CCC	CTC	CTC	TGG	TCC	CCC	CAG	AGG	CGG	GAA	GGC	CCC	Clar	Tou	Pho	Δla	2300
		Leu			615					650					033		2556
	CGC	ACC	CAC	GGC	CAG	CCC	GGC	TAC	TTC	TAC	Clu	LTC	FOT	Tou	Acn	Pro	2550
20	Arg	Thr	His	Gly	GIn	Pro	GIY	TYT	665	TYL	GIU	Deu	361	670	nap	110	
20	000	TTC	mac	660	CTC	CTC	CTC	GCC	CGC	СТТ	AAG	GAG	GGG		GAG	GGG	2604
	01	Phe	TWE	Ara	Leu	Leu	Leu	Ala	Ara	Leu	Lvs	Glu	Gly	Phe	Glu	Gly	
	_		675					680					685				
	CGG	AGC	CTC	CGG	GCC	TAC	TAC	CGC	GGC	CGC	CAC	CCG	GGT	CCC	GTG	CCC	2652
25	Ara	Ser	Leu	Arg	Ala	Tyr	Tyr	Arg	Gly	Arg	His	Pro	Gly	Pro	·Val	Pro	
25	_	600					695					700					2700
	GAG	GCC	GTG	GAC	CTC	CTC	CGG	CCC	GGA	CTC	GCG	GCG X 1 o	Clar	GAG	GUG	Val	2700
		Ala	Val	Asp	Leu	Leu	Arg	PTO	GIY	Leu	715	ATG	GLY	Giu	GLY	720	
•	705	GTC	~.~	C.T.C	000	710	CTC	C A A	CAC	GGG	GGC	CTG	GAC	CGC	ACG		2748
30	TGG	Val	CAG	CTC	Clv	LTC	Val	Gln	ASD	Glv	Glv	Leu	Asp	Ara	Thr	Glu	
30		GTC			725					730					/33		2796
	CGG	GTC Val	CTC	CCC	CGC	CTG	BAC	Lau	Bro	Trn	Val	Leu	Arg	Pro	Glu	Glv	
	Arg	Val	Leu	740		reu	Meh	Deu	745	LLP	V	204		750		•	
	CCC	CTC	ጥጥር	TCC	CAG	CGG	GGC	GCC	TCC	AGA	AGG	GTC	CTC	GCC	CTC	ACG	2844
35	Glv	Leu	Phe	Trp	Glu	Arg	Gly	Ala	Ser	Arg	Arg	Val	Leu	Ala	Leu	Thr	
			755					760					700				2002
	GGA	AGC	CTC	CCC	CCG	GGC	CGC	CCC	CAG	GAC	CTC	TTC	GCC	GCC	CTG	CAG	2892
	_	Ser 770					775					780					2940
40	GTC	CGG	CTC	CTG	GAA	AGC	CTT	CCC	CGC	CTC	CGG	GGG	CAC	GCC	CCC	Clar	2940
	725	Arg				790					795					800	2988
	ACC	CCA	GGC	CTC	CTT	CCC	GGG	GCC	CTG	CAC	GAG	ACC	GAA	310	TOU	Unl	2900
45		Pro			805					810	l				812		2026
45	CGC	CTC	CTC	GGG	GTG	CGC	CTC	GCC	CTC	CTC	CAC	CGG	GCC	CTT	. C1	Clu	3036
	_			820					825					830	,	Glu	2084
	GTG	GAG	GGG	GTG	GTG	GGG	GGC	CAC	CCC	CTC	CTA	GGC	CGC	GGC	CTC	C1++	3084
50			835					840	)				845			Gly	3132
	GCC	TTC	CTG	GAG	CTG	GAG	GGG	GAG	GTG	TAC	CTC	GTG	GCC N 1 ~	UTG	GGC	Ala	JIJZ
	Ala	Phe	Leu	Glu	Leu	Glu	Gly	GIU	val	тук	ren	. Agl	WTS	ושעו	. Gry	Ala	

	GAA Glu	850 AAG Lvs	CGG Arg	GGC Gly	IIIT	•					875				-	Asp 880	3180
5	065		CGG Arg		GTG Val	CAC His	CTC Leu	GCC Ala	CTC Leu	GAG Glu	GCC Ala	CTG Leu	GAG Glu	GCG Ala	GAG Glu 895	Leu	3226
	TGG	GCC Ala	TTT Phe	GCC Ala	GAG Glu	GAG Glu	GTG Val	GCC Ala	GAC Asp	CAC His	CTC Leu	CAC His	GCC Ala	GCC Ala 910	Phe	CTC Leu	3324
10			TAC	CGC Arg	TCC Ser	GCC Ala	CTC Leu	CCC Pro	GAG Glu	GAG Glu	GCC Ale	CTG Leu	GAG Glu 925	Glu	Ala	GGC Gly GAG	3372
, 15	тGG	ACG	CGG	CAC	ATG Met	GCC Ala	GAG Glu	GTG Val	GCG	GCG Ala	GAG	His	Leu	His	Arg	Glu	3420
							CGC									GCC Ala 960	3429
20	945 GG	S AAA	A GCC	:								a mm/		rcGA	AGλ	CGGCCTC	3489
	CT	CGGG	CCCG GAGG CACC	AGG	CCCT CGGCC CCCG	<b>3</b> C 1	GCC( CTT( CGGG(	CCGG( GCC( GTAG(	SC CA	CACC	AGAC GCTC	G GCG	TCC(	CACA	TGCC	EGGCCTC EGCAGA	3549 3600
25																	

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH:39 base pairs
- (B) TYPE:nucleic acid
- (D) TOPOLOGY:linear
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

AGCTTGAATT CTTTTTTAAT AAAATCAGGA GGAAAAACC

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#### Claims 40

- 1. A recombinant enzyme which is capable of converting maltose into trehalose and not substantially inactivated even when incubated at a temperature of over 55°C.
- 2. The recombinant enzyme of claim 1, which has the following physicochemical properties: 45

Forming trehalose when acts on maltose, and vice versa;

About 100,000-110,000 daltons when assayed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (2) Molecular weight (MW) (SDS-PAGE);

(3) Isoelectric point (pl)

About 3.8-4.8 when assayed on isoelectrophoresis;

(4) Optimum temperature

About 65°C when incubated at pH 7.0 for 60 min;

(5) Optimum pH

About 6.0-6.7 when incubated at 60 C for 60 min;

(6) Thermal stability

Stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min; and

Stable up to a pH of 5.5-9.5 even when incubated at 60°C for 60 min.

The recombinant enzyme of claim 1, which has the amino acid sequences of SEQ ID NOs:1 and 2 as a partial amino acid sequence:

## SEQ ID NO:1:

Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val 10 Arg Ser Phe Phe 20

#### SEQ ID NO:2: 15

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Ile Leu Leu Ala Glu Ala Asn Met Trp Pro Glu Glu Thr Leu Pro 1

The recombinant enzyme of claim 1, which has an amino acid sequence selected from the group consisting of the amino acid sequence in SEQ ID NO:3, and homologous amino acid sequences thereunto:

SEQ ID NO:3: Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val Arg Ser Phe Phe Asp Ala Asn Asn Asp Gly Tyr Gly Asp Phe Glu Gly Leu Arg Arg Lys Leu Pro Tyr Leu Glu Glu Leu Gly Val Asn Thr Leu Trp Leu Met Pro Phe Phe Gln Ser Pro Leu Arg Asp Asp Gly Tyr Asp 30 Ile Ser Asp Tyr Tyr Gln Ile Leu Pro Val His Gly Thr Leu Glu Asp Phe Thr Val Asp Glu Ala His Gly Arg Gly Met Lys Val Ile Ile Glu 35 Leu Val Leu Asn His Thr Ser Ile Asp His Pro Trp Phe Gln Glu Ala Arg Lys Pro Asn Ser Pro Met Arg Asp Trp Tyr Val Trp Ser Asp Thr Pro Glu Lys Tyr Lys Gly Val Arg Val Ile Phe Lys Asp Phe Glu Thr 40 Ser Asn Trp Thr Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg Phe Tyr Trp His Gln Pro Asp Leu Asn Trp Asp Ser Pro Glu Val Glu 45

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Lys Ala Ile His Gln Val Met Phe Phe Trp Ala Asp Leu Gly Val Asp

	. 100
	180 185 180 180 180 180 180 190 190 190 190 190 190 190 190 190 19
	180 Pen Ala Ile Pro Tyr Leu Tyr Glu Alg 325
	Gly Phe Arg Leu Asp Ala 11e Plo 112 205  200  195  Ser Cys Glu Asn Leu Pro Glu Thr Ile Glu Ala Val Lys Arg Leu Arg  220  220  215  220  220  230  240
5	Ser Cys Glu Asn Leu Pro Glu IIII 220 220 215
	710 - mass CIV PIO G11 -2 - 240
	Lys Ala Leu Glu Glu 230 235 Phe Gly Asp Gly Asp
10	
,,,	Ala Asn Met Trp Pro Glu Glu Thr Leu 250 Ala Asn Met Trp Pro Glu Glu Thr Leu 250  245  Gly Val His Met Ala Tyr Asn Phe Pro Leu Met Pro Arg Ile Phe Met 270  265  Cly Pro Ile Glu Thr Met Leu Lys Glu
	Ala Leu Arg Arg Glu Asp Arg Gly 280 280 275 Ala Glu Gly Ile Pro Glu Thr Ala Gln Trp Ala Leu Phe Leu Arg Asn 300 295 Ala Glu Gly Ile Pro Glu Thr Glu Glu Glu Arg Glu Phe
15	Ala Glu Gly Ile Pro Glu Thr Ala Glu 300
13	7UI) 61. THE VAL **** 7-* 320
	Ala Glu Gly Ile Pro Glu Thr Ala 300 295 290 His Asp Glu Leu Thr Leu Glu Lys Val Thr Glu Glu Glu Arg Glu Phe 310 320 320 310 310 310 310 300 315 310
20	Ile Arg Arg Leu Met Pro Leu 345 345 Glu Leu Leu Thr Ala Leu Leu Leu Thr Leu Lys Gly Thr Pro Ile Val 365 360 360 365 360 360 360 360 360
	Glu Leu Leu Thr Ala Leu Leu Leu Gly Asp 360 355  Tyr Tyr Gly Asp Glu Ile Gly Met Gly Asp Asn Pro Phe Leu Gly Asp 380 375 370  Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val 395  Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val 395  Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val 395
25	
	Arg Asn Gly Val Arg 390 390 Leu Phe Leu Pro Pro Val Ser
	Arg Asn Gly Val Arg Thr Pro Met Gli 395 385 Ala Phe Ser Arg Ala Pro Tyr His Ala Leu Phe Leu Pro Pro Val Ser 415 410 405 410 405 410 405
30	Glu Gly Pro Tyr Ser Tyr Man 425
	433 BLE CITY AND GLY SOL
	Asn Gln His Ala Lys Ite File 501 450 Asn Glu His Glu Gly Glu
35	
	Arg Val Leu Val Val Ala Ash hed 490
40	Leu Pro Leu Giu Ang Tyr Arg Leu Thr Leu Giy
	Leu Pro Leu Glu Ala Tyr Gin Gly 505 500 500 Gln Gln Pro Phe Pro Pro Val Glu Gly Arg Tyr Arg Leu Thr Leu Gly 525 520 520 520 520 520 520 520 520 520
	Gln Gln Pro Phe Pro Pro Val Glu Ala Val Leu  515  Pro His Gly Phe Ala Leu Phe Ala Leu Lys Pro Val Glu Ala Val Leu  530  His Leu Pro Ser Pro Asp Trp Ala Glu Glu Pro Ala Pro Glu Glu Val  550  550  Trp Gly Gly Pro Glu Val Leu Leu Val
45	
	His Leu Pro Ser Pro Asp Trp Ald 555 550 545 Asp Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu Val 575 Asp Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu Asn Ala Leu 590 Asp Thr Leu Val His Glu Arg Gly Arg Glu Glu Leu Leu Asn Ala Leu 590 585 585
	ALL AND GIV AID OLD FOO
50	THE TAKE COT TILL DOW
	Asp Thr Leu Val His Glu Arg 517 585 580 580 605 600 Rhs Cln Lys Asp Pro Pro Leu Tyr
	333 Ara Phe Gin 22
	Val Ala Leu Leu Asp 615 615 The Leu Gln Val Ser Leu
£	Val Ala Leu Leu Asp Ala Leu Arg 500 620 620 615 610 610 Leu Thr Leu Gln Leu Glu Asn His Arg Thr Leu Gln Val Ser Leu
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	640
	635 630 630 625 Pro Leu Leu Trp Ser Pro Gln Arg Arg Glu Gly Pro Gly Leu Phe Ala 655 Pro Leu Leu Trp Ser Pro Gln Arg Arg Glu Leu Ser Leu Asp Pro 645 670
	625 men Ser Pro Gln Arg Arg Glu Gly 120 655
	pro Leu Leu Trp 5645 645 Glu Leu Ser Leu Asp 120 670
5	Pro Leu Leu Trp Ser Pro Gln Arg Arg 650  Pro Leu Leu Trp Ser Pro Gln Arg 650  645  Arg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro 670  665  Arg Leu Lys Glu Gly Phe Glu Gly 665
	Arg Thr His Gly Gln Pro Gly Tyr Phe 192 670  665  660  Gly Phe Tyr Arg Leu Leu Ala Arg Leu Lys Glu Gly Phe Glu Gly  685  680  680  680  680  687  687  687  687
	675 Arg Gly Arg His Pro Gly F10
	Gly Phe Tyr Arg Leu Leu Leu Ala Arg Leu I G85 680 675 Arg Ser Leu Arg Ala Tyr Tyr Arg Gly Arg His Pro Gly Pro Val Pro 690 Glu Ala Val Asp Leu Leu Arg Pro Gly Leu Ala Ala Gly Glu Gly Val 720 715 710
10	715
	7/05 - 725
	Val Gln Leu Gly Leu Val Gln Asp 021 730 730 Pro Glu Gly
15	725 Trp Val Leu Asp Leu Pro Trp Val Leu Arg 750
	Trp Val Gln Leu Gly Leu Val Gln Asp Gly 730 725  Arg Val Leu Pro Arg Leu Asp Leu Pro Trp Val Leu Arg Pro Glu Gly 750 745 740  Gly Leu Phe Trp Glu Arg Gly Ala Ser Arg Arg Val Leu Ala Leu Thr 765 760 7757  7758 7759 7759 7750 7750 7750 7750 7750 7750
	Gly Leu Phe Trp Glu Arg Gly Ala Sel Arg 765 760 755 Gly Ser Leu Pro Pro Gly Arg Pro Gln Asp Leu Phe Ala Ala Leu Glu 775 775 775 777 778 779 770 770 770 770 770 770 770 770 770
20	755 Pro Pro Gly Arg Pro Gln Asp Leu 780
20	Gly Ser Leu Pro Pro Gly Arg Pro Gli Asp 780 775 770 770 Val Arg Leu Leu Glu Ser Leu Pro Arg Leu Arg Gly His Ala Pro Gly 795 790 Val Arg Leu Leu Glu Ser Leu Pro Arg Leu His Glu Thr Glu Ala Leu Val 790 815
	Val Arg Leu Leu Glu Ser Leu Pro Arg Leu 795  Val Arg Leu Leu Glu Ser Leu Pro Arg Leu 795  785  Thr Pro Gly Leu Leu Pro Gly Ala Leu His Glu Thr Glu Ala Leu Val  815  Thr Pro Gly Leu Leu Pro Gly Ala Leu Leu His Arg Ala Leu Gly Glu  805
	785 Bro Gly Leu Leu Pro Gly Ala Leu 810 810 Arg Ala Leu Gly Glu
25	805 Rev Val Arg Leu Ala Leu Leu His Arg 120 830
	Thr Pro Gly Leu Leu Pro Gly Ala Leu R10 810 805 Arg Leu Leu Gly Val Arg Leu Ala Leu Leu His Arg Ala Leu Gly Glu 825 826 827 828
	Val Glu Gly Val Arg Leu Ala Leu Bed
30	835 She Leu Glu Glu Glu Val Tyr B60 She Leu Glu Leu Glu Glu Val Tyr Asp
•	Ala Phe Leu Glu Leu Glu Gly Glu Val 192 860  Ala Phe Leu Glu Leu Glu Glu Asp Leu Ala Arg Leu Ala Tyr Asp  850  Glu Lys Arg Gly Thr Val Glu Glu Asp Leu Glu Ala Glu Leu  870  870  870  895
	Glu Lys Arg Gly Thi 870 870 Leu Glu Ala Glu Leu 895
	Glu Lys Arg Gly Thr Val Glu Glu Asp Leu 875  Glu Lys Arg Gly Thr Val Glu Glu Asp Leu Glu Ala Glu Leu  865  Val Glu Arg Ala Val His Leu Ala Leu Glu Ala Leu Glu Ala Glu Leu  895  Val Glu Arg Ala Val His Leu Ala Asp His Leu His Ala Ala Phe Leu  910
3	Val Glu Arg Ala Val His Leu Ala Leu Glu 890  Val Glu Arg Ala Val His Leu Ala Leu Glu His Ala Ala Phe Leu  Trp Ala Phe Ala Glu Glu Val Ala Asp His Leu His Ala Ala Phe Leu  905  Trp Ala Phe Ala Glu Glu Val Ala Leu Glu Glu Ala Gly  900
	Trp Ala Phe Ala 900 905 905 905 925
	Trp Ala Phe Ala Glu Glu Val Ala Asp 115 910 905 900 905 900 Glu Glu Ala Leu Glu Glu Ala Gly 925 920 920 915 925 Trp Thr Arg His Met Ala Glu Val Ala Ala Glu His Leu His Arg Glu 940 935 935 935 930 930 935 936 960
	915 940 940 940 940 940 940 940 940
	Trp Thr Arg His Met Ala Glu Val Ala Ala 940 935 930 930 Glu Arg Pro Ala Arg Lys Arg Ile His Glu Arg Trp Gln Ala Lys Ala 950
	Glu Arg Pro Ala 950
	Gly Lys Ala
	45 of claim 1.

5. A DNA which encodes the recombinant enzyme of claim 1.

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6. The DNA of claim 5, which has a base sequence selected from thr group consisting of the base sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary base sequences to these base sequences. 50

	SEQ ID NO:4:  GTGGACCCCC TCTGGTACAA GGACGCGGTG ATCTACCAGC TCCACGTCCG CTCCTTCTT TCCCTACCTG 120  180  180  180  240
	TCCCTACCA GGACGCGGTG ATCTACCAGC TCCCAGCCT TCCCTACCTG 120
	GTGGACCCCC TCTGGTACAA GGRGGACTTT GAGGGCCTGA GGCGGACCCCC CTTGAGGGAC 240
5	SEQ ID NO:4:  GTGGACCCC TCTGGTACAA GGACGCGGTG ATCTACCAGC TCCACGTCCG CTCCTACCTG 120  GACGCCAACA ACGACGGCTA CGGGGACTTT GAGGGCCTGA GGCGGAAGCT TCCACGGGAC CTTGAGGGAC CCTTGAGCAG ATCCTCCCG TCCACGGGAC CCTTGAGCAG 300  GACGGGTACG ATATCTCCGA ATCTCCAG ATCACAGG ATGAAGGTGA ATCCTCCCG TCCATGAGC CCCCATGCGG 360  ACGAGGCCAA ACGAGGCCCA CGGCCGGGGG ATGAAGGTGA AGCCGAATAG CCCCATGCGG 360  ACGAGGCCCA ACGAGGCCCA CGGCCGGGGG ATGAAGGTGA AGCCGAATAG CCCCATGCGG 420
•	GAGGAGCTCG GGGTCAACAC CCTACTACCAG ATCCTCCCCG TCCACGAGCT CGTCCTGAAC 300
	GAGGAGCTCG GGGTCCACCACCACCACCACCACCACCACCACCACCACCACCA
	GACGGGTACG ACGAGGCCCA CGGCCGGGGG ATGGCGAGGA AGCCGAATAG CCCCTTCAAG 420 TTCACCGTGG ACGAGCCACCC TTGGTTCCAG GAGGCGAGGA AGCCGAATAG CATCTTCAAG 420 TTCACCACCG ACGAGCCACCC TTGGTTCCAG GAGGCGAGGA AGCCGAATAG CATCTTCAAG 420 TTCACCACCG
	CACACCTCCA TTGACCACCC TTGGTAGAGG AAGTACAAGG GGGTCCTACTA CTGGCACCGC 480  CACACCTCCA TGTGGAGCGA CACCCCGGAG AAGTACAAGG GGGTCCTACTA CTGGCACCGC 540
	CACACCTOOL TGTGGAGCGA CACCCOTTGAC CCCGTGGCCA AGGCCTAGAA GGCCATCCAC 540 GACTGGTACG TGTGGAGCGA CACCCCG AGGTGGAGAA GGCCATCCAC 600
10	THE TARREST TO STATE OF THE PROPERTY OF THE PR
	GACTTTGAAA CCTCCAACTGG GACAGCCCTGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCTGGA CCGCCCGAGACCAT TGAGGCCGGT 720 CAGGTCATGT TCTTCTGGGC CGACCTCCTGC GAGAACCTCC CCGAGACCAT TGAGGCCGAG 720 CAGGTCATGT TCTTCTGGGC CGACCTCTGC GAGAACCTCC CCGAGACCAT TCTTCCGGA GCGCCCACATG 780 ACCCCCTCAACTGG GACCCCCCC TACCTCCCCAACTG GGACCGGGGT 840
	TTCTACTGC ACCAGCOCT GGGGG GTGGACCTC CCGAGACCAT TGAGGCGCGGG 720 CAGGTCATGT TCTTCTGGGC CGACCTCCTC GAGAACCTCC CCGAGACCAT TGAGGCGCGAG 720 CAGGTCATGT ACCGGGAGGG GACCTCCTCC GAGAACCTC CCTCGCCGAG 780 TACCTCTACG GGAAGACCCT TACGGCCC TACGGGGACGA GGACCACATG 780 AAGCGCCTGA GGAAGACCCT GGAGGAGCGC TACTTCGGGG ACGGGGACGA GGACCGGGGT 840 AAGCGCCTGA GGCCGGAGGA GACCCTCCCC TACGCCGGAGACCATC TTCATGCCCC TAAGGCGCAACCACCC 900
	TACCTCTACG AGCGGAGGG GACCTACGGCCCCG GGAAGACGG GGTCCACATG TACCTCTACG AGCGCCCCT GGAGGAGCGC TACGGCCCCG ACGGGGACGG GGTCCACATG 840
	CAGGTCATGT TCTCTCTGC GAGAGCCCCG GGAAGATCCT CCTCGCGCTATG TACCTCTACG AGCGGGAGG GACCTCCTGC TACGGCCCCG GGAAGATCCT CCTCCACATG AAGCGCCTGA GGAAGGCCCT GACCTCCCC TACGGCGCGGA GGACCGGGGT 840 GCCAACATGT GCCCCGGATC TTCATGGCCC TAAGCCGCCCA GTGGGCCCTC 900 GCCTACAACT TCCCCCTGAT GCCCCGGAGGAGGACGA GCGGGAGTTC GCCTACAACT CCCCCTCAA GGAGGGGGAGA GGACCGCGCG GAGAAGCCCCCA GCGGGAGTTC 1020
15	AAGCGCCTGA GGAAGGAGA GACCCTCCCC TACTTCATGCCC TAAGGCGGGA GGACCGCCTC 900 GCCAACATGT GGCCGGAGGA GACCCTGCCC TACTTCATGCCC TAAGGCGGGA GGACCGCGCCTC 900 GCCAACATGT TCCCCCTGAT GCCCCGGATC TTCATGCCCCA AAACCGCCCA GTGGGCCCTC 960 GCCTACAACT TCCCCCTGAT GCGGAGGAGGA GCGCGGAGGTTC GAGAAGGTCA CCGCGGGAGTTC CCCCCTCCTCCTC 1080
•-	GCCAACATGT GGGGCCCGGATC TICHTOCCCCA AAACCGCCCA GIGGGAGTTC 960
	GCCTACATA CCATGCTCAA GGAGAGGTCA CGGAGGAGT CCGCCGCCGC 1020
	GCCAACATGT GGCCCGGATC TTCATCCCCA AAACCGCCCA GTGGGCCTCA GCCTCATGAAA CCATGCTCAA GGAGGCGGAG GGGATCCCCG AAACCGCCCA GCGGAGGTTC GGGATCCCCG CCATTGAAA CCATGCTCAA GCCACCCAAG GCTCACCCAAG CCTACGCCCC CGACCCCAAG TTCCGCATCA ACCTGGGGAT CCTCCTCCTC 1080 CTCATGCCCC CCTCCTCCCC CGACCCCAAG CCGCTACGACG CGGTACGAGA TCCGCATGGA CCGCATCGTC 1200 CTCATGCCCC CGCCCCCAT CCTCCTCCTC CCCATGCACG CGCCCCCAAG CCCCATGCAC CCGCCTCCCTC 1200 CCCCATGCCC CCCCCCCATGCAC CCCCATGCAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACAC CCCCATGCACACAC CCCCATGCACACAC CCCCATGCACACAC CCCCATGCACACAC CCCCATGCACACACAC CCCCATGCACACAC CCCCATGCACACACACACACACACACACACACACACACAC
	TTCCTCCGCA ACCACGACGA GGACCCCAAG TTCCGCATCA TCCTCACCGC CCTCCTCCTC 1000 ATGTACGAGG CCTACGCCCC. CGACCGCAGG CGGTACGAGC TCCTCACCGC GGACAACCCC 1140 ATGTACGACG CCTCTCGGGGG CGACCGCAGG CGGGACGAGA TCGGCATGGG GGACAACCCC 1200 CCTCATGCCCC TCCTCGGGGG CGGCCCAT CCTCACGCAGGACC CCCATGCAGT CCCCTAGGACCG CCGTGAGCGA CCGCATCCTC TTCCTTCCCC CCGTGAGCGA CCCTGAGCTTC 1320
	ATGLACGCC TCCTCGGGGG CGACGACTAC GGGGACGAGA TCGGCALAGA CCGCATCGTC 1200
20	
	ATGTACGAGG CCTACGCCCC CGACCGCAGG CGGTACGAGC TCGGCATGGG GGACAACCCC 1120 CTCATGCCCC TCCTCGGGGG CGACCGCAGG CGGGACAAGA TCGGCATGGG GGACAACCCC 1200 ACCCTAAAGG GCACGCCCAT CGTCTACTAC GGGGACGAGA CCGCATGCAGA CCGCATGCAGA CCGCATGCAGA CCGCTAC 1260 TTCCTCGGGG ACCGGAACGG TGTCAGGACC CCCATGCCCT CCGTGAGCTTC 1320 CCCCCCCCAG CGGGAAAACC CCCACTCCCT CCCACTCCCT 1380
	CTCATGCCC TCCTCGGGGG CGTCTACTAC GGGGACGAGA TCGCCAAGA CCGCATCGTC 1260 ACCCTAAAGG GCACGCCCAT TGTCAGGACC CCCATGCAGT CCGTGAGCGA GGGGCCCTAC 1260 TTCCTCGGGG ACCGGAACGG TGTCAGGACC CCCATGCCCT CCCACTCCCT CCTGAGCTTC 1320 GCCTTCTCCC GCGCCCCCTA CGGGAAAACC CCCACTCCCT CCCACTCCCT TCGTCAACGT TCGTCAACGT CACGCCAAGA TCTTCGGCCG GGGGAGCCTC 1380 GCCTTCTCCC TCGTCAACGT CACGCCAAGA TCGTCAGCACG CGAGGGGGAG 1440
	ACCCTAAAGG GCACGCCCAT CGTCAGGACC CCCATGCAGT GGTGAGCGA GGGGCCCTAC 1200 TTCCTCGGGG ACCGGAACGG TGTCAGGACC CCCATGCCCT CCTGAGCTC 1320 GCCTTCTCCC GCGCCCCTA CCACGCCCTC CCCACTCCCT CCTGAGCTC 1380 GCCTTCTCCC GCGCCCCTA CCACGCCCAGA TCTTCGGCCG GGGGAGCCTC 1380 AGCTACCACT TCCTCGCCCT GGAGGACCAG CACGCCAAGA TCTTCGGCCG CGAGGGGGAG 1440 AGCTACCACT TCCTCGCCCT GAGGACCAC CTCGCCTACC CCCCTTGGAG 1500
	TTCCTCGGGG ACCGGAACGG TGTCCTCCCC CCCACTCCCT CCTGAGCTTC 1380 GCCTTCTCCC GCGCCCCTA CCACGCCCAG CCGGGAAAACC CCCACTCCCT GGGGAGCCTC 1380 ACCGCCGCT TCCTCGCCCT GAGGAACCAG CACGCCAAGA TCTTCGGCCG CGAGGGGGAG 1440 AACCGCCGCT TCCTCGCCCT GAGGAACCAG CTCGCCTACC TACACCCAGG CCCTTGGAG 1500 CCCCTTGGAGAA CCCCTTGGAGA 1560 CCCCTTGGAGAA CCCCTTGGAGA 1560
25	GCCTTCTCCC GCGCCCCTA AGCTACCACT AGCTACCACT ACCGCGCT ACCGCGCCCCTA ACCGCCGCT ACCCCTTCTCCC CCGTGGAGAA CCCTTCTCCC CCGTGGAGAA CCCTCTCCCCC CCGTGGAGAA CCCTTCCCCC CCGTGGAGAA CCCTTCCCCC CCGTGGAGAA CCCTTCCCC CCGTGGAGAA CCCTTCCCCC CCGTGGAGAC CCTCTCCCCC CCGTGGAGCC CCTCTCCCCC CCGTGGAGCC CCTCTCCCCC CCGTGGAGCC CCCTTCCCCC CCGTGGAGCC CCCTTCCCCC CCGTGGAGCC CCCTTCCCCC CCGTGGAGCC CCCTTCCCCC CCGTGGAGCC CCCCTTCCC CCGTGGAGCC CCCCTCCCC CCGTGGAGCC CCCCTCCCC CCGTGGAGCC CCCCTTCCCC CCCCTCCCCC CCGTGGAGCC CCCCTCCCC CCCCTCCCCC CCCCCCCCCC
	ACCCTTCTCC CCGTGGAGAA CCCTCCCGC TACACCCAGG CCTCCCC CCCGGTGGAG 1500  CGGGTCCTGG TGGTGGCCAA CCTCTCCCGC TACACCCAGG AACCCTTCCC CCCGGTGGAG 1620  CGTGGAGCTC TCTCCCCCT GAAGCCCGTG 1620  CGTGGACCAC GGCTCGCCC TCTTCGCCCT GAAGCCCGTG 1680  CGCTACCAAG GCCTCGCCC GGGCCCCCCAC GGCTTCGCCC CCGAGGAGGCC 1680  CCTCCCCCCGGAC TGGGCCGAGG AGCCCGCCCC CACCCTGGTC 1740  CCTCCCCCCGGAC CCCGGAGGTCC TCCCCCCGAC TGGGCCGAGGAGAGAGC 1800
	CGGGTCCTGG TGGTGGCCAA CGTGGAGCTC TTCTCGCAGC AGACCCCGT GAAGCCCGTG 1680 GCCTACCAAG GCCTCGTCC GGGCCCCAC GGCTTCGCCC TCTTCGCCCT GAAGCCCGTC 1680 GGGCGCTACC GCTTGACCCT CCCCCGAC TGGGCCGAG AGCCCGCCC CACCCTGGTC 1740 GGGCGGTGC TCCACCTCCC CTCCCCGAC CCGGGGGG CCCGGGGGG CCGGGGGGC CCGGGGGGC CCGGGGGG
	GCCTACCAAG GCCTCGTCCC GGGCCCCAC GGCTTCGCCC CGAGGAGGCC 1740 GGGCGCTACC GCTTGACCT GGGCCCCAC TGGGCCGAGG AGCCCTGGTC 1740 GAGGCGGTGC TCCACCTCCC CTCCCCGAC TGGGCCGAGGTCC TCCTGGTGAA GGAGAAGAGC 1800 GACCTGCCCC GGGTCCACAT GCCCGGGGGG GCCCTCGCCC AGACCCTGAA GGAGAAGAGC 1860 GACCTGCCCC GGCGGAGGAG GCCCTCGGCC CTCCTGGACG CCCAGAAGGAC 1920 GCCTCCGCCC CTCCTGGACG CCCCTCCGCTT CCAGAAGGAC 1920
	GGGGGGTACC GCTTGACCT CTCCCCGAC TGGGCCGAGG ACCCCTGGTC 1800 GAGGCGGTGC TCCACCTCCC CTCCCCGAC TCCTGGTGAA GGAGAAGAGC 1800 GACCTGCCC GGGTCCACAT GCCCGGGGGG GCCCTCGCCC AGACCCTGAA GGAGAAGGAC 1860 CACGAAAGGG GGCGGGAGGA GCTCCTAAAC GCCCTCGGCC CCCTCCGCTT CCAGAAGGAC 1920 CACGAAAGGG GGCGGAGGA GAAGGTGGCC CTCCTGGACG GGACCCTCCA GGTCTCCCTC 1920 CACGAAAGGG CTCCCGCC GAGAACCACA GGACCCTCCA GGTCTCCCTC 1980
	GAGGCGTGC GAGGCGTGC GAGGCGGAGA GCCCGGGGGG GGCCCCCCC GGGTCCACAT GCCCCTCAAAC GCCCTCGCCC GGGTCCACAT GCCCCTCAAAC GCCCTCGCCC CCAGAAGGAC CCCAGAAGGAC CCCAGAAGGAC CCCACACGCC TCAAGCCGCA GAAGGTGGCC GAGAACCACCA GGCCCCGGCC TCACCCCCCC CCCCCCCCCC
30	GACCTGCCC GGGTCCACAT GCCCTAAAC GCCCTCGCCC ACCAGAAGGAC 1920 CACGAAAGGG GGCGGGAGA GCTCCTAAAC CCCCTCGCCT CCAGAAGGAC 1920 CACGAAAGGG GGCGGGAGA GAAGGTGGCC CTCCTGGACG CCCCTCCCACCT 1920 TGGCTCGCCC TCAAGCCGCA GAAGGTGGCC GAGAACCACA GGACCCTCCACCGC 1980 CCGCCCCTTT ACCTCACCCT GCCCCCA GGCCCCGGCC TCTTCGCCCGC CCTCCTCGCC 2040 CCGCCCCTCT GGCCCCCCA GGCCCCCGGCC TCTTCCGCCCG CCCCCCCCCC
	CACGAAAGGG GGCGGGAGA GAAGGTGGCC CTCCTGGACG GGACCCTCCA GGTCTCCTC 1980 TGGCTCGCCC TCAAGCCGCA GCTCCAGCTG GAGAACCACA GGACCCTCCA CACCCACGGC 1980 CCGCCCCTTT ACCTCACCCT GAGGCGGGAA GGCCCCGGCC TCTTCGCCCG CACCCACCGC 2040 CCCCTCCTCT GGTCCCCCCA GAGGCGGGAA GCCCAGGCT TCTACCGCGC CCGCCACCCG 2100 CCCCTCCTCT ACTTCTACGA GCTCTCCTTG GACCCAGGCT ACTTACCGCGG GGAGGGGGTC 2160
	COGCCCTTT ACCTCACCCT CACCTGGGAA GGCCCCGGCC TCTACCGCCT CCTCCTCGCC 2100
	CCGCCCTTT ACCTCACCCI GAGGCGGGAA GGCCCCGGCC TCTACCGCCT CCTCCTCGC 2100 CCCCTCCTCT GGTCCCCCA GAGGCGGAA GGCCCAGGCT TCTACCGCCT CCGCCACCCG 2100 CAGCCCGGCT ACTTCTACGA GCTCTCCTC GACCCAGGCCT ACTACCGCGG GGAGGGGGTC 2160 CAGCCCGGCT ACTTCTACGA GGGCCGGACC CCGCCACCCC 2220 CCGCCTTAAGG AGGGGTTTGA GGGACCTCCTC CGGCCGGGAC TCGCGGAGGCG GGTCCTCCCC 2220 CCGCCTTAAGG AGGGCCGT GGACCTCCTC CGGCCGGGACC GCGCGGGCCC 2280 CCCCCTCTCCCC CCGAGGCCGT GGACCTCCTC CGGCCGGGACC GCGCGGGCCC 2280
	CAGCCGGCT ACTTCTACGA CCGGCGGAGC CTCCGGGCCT MCGCGGCGGG GGAGGGGGTC 2120
3	CAGCCCGGCT ACTTCTACGA GGGCCGGAGC CTCCGGGCCG GGAGGGGGGGGGG
	CCTCCGTGC CCGAGGCCGT CCAAGACGGG GGCCTGGACC MCTTCTGGGA GCGGGGCGCC 2340
	TEGETCUAGE 1000 TOTCEGGCCC OILL TOTCEGGCCCCCCA GOLLG
	CGCCTGGACC TOTAL CACGGGAAGC OTTAL TOTAL TOTAL TOTAL TAGGC TAGGGGGGA COOTTAL TAGGC TAGG
	TCCAGAAGGG TCCTCGCCT CCTGGAAAGC CTTCCCCGC CCTCGTCGG CCTCCTCGGG 2520
	GCCGCCCTGG AGGTCCGGC GGCCCTGCAC GAGACCGAAG CCCTGGT GGGGGGCCAC 2580
	ACCUAGGO CCGGGCCCII OOO ACCGGGGAGGI O
	TCCAGAAGGG TCCTCGCCCT CCTGGAAAGC CTTCCCCGCC CCTCCTCGGG 2520  GCCGCCCTGGG AGGTCCGGCT CCTGGAAAGC CCTGGTCCG GGGGGGCCAC 2520  ACCCCAGGCC TCCTCCCA CCGGGCCCTT CCGGGGCCTTC CTGGAGCTGG AGGGGGAGGT GTACCTCGTG 2580  CCCCTCCTCCAG CCGGGCCTT CCGGGGCCTT CCGGGGCCTT CCGGGCCCTT CCGGGCCCTT CCGGGCCCTT CCGGGCCCTT CCGGGCCCTT CCGGGCCTTTGCC 2760  CCCCTCCTCGG AGGCCCTC CCGGGCCAC CCGGGGCCAC CCGCCTCCCC 2760
	CCCTCCTAG GCCGCGCGCGCGGGGGGGGGGGGGGGGGGG
	ACCCAGGCC TCCTCCTCA CCGGGCCCTT GGGGAGCTGG AGGGGGAGGT GTACCTCGCCGGGCCTCGAGGCCTTC CTGGAGCTGG AGGGGGAGGT GGCCTACGAC 2640 CCCCTCCTAG GCCGGGCCTT CTGGAGGAGGACC TGGCCCGCCT GGCCTTTGCC 2700 GCCCTCGAGG GCCCTGGAGG GCCCTGGAG GCCCTCCCC 2760 CCCCCTCGAG CCCCTCCCC CCCCCCCC CCCCCCCC CCCCCCCCC CCCCCC
	GTGCGCCTCG CCCTCCTCA CCCCTCCTCA CCCCTCCTAG CCCCTCCTAG CCCCTCCTAG CCCCTCCTAG CCCCTCCTAG CCCCTCGAG CCCCTGGAGC CCGGAAAAGCG CCGGCACCACCT CCGCCCTCGAG CCCCTGCAGC CCGCCCTCGAG CCCTCCCCC CCGCCCTCCAG CCCTCCCCC CCGCCCTCCCC CCGCCCCCCC CCGCCCCCCCC
	GAGGAGGTGG CCGACCACCT CONTROL CGGCACATGG CCGACCTGGCA GGCCAAGGCC 2889
	GAGGAGGCCC TGGAGGAGGC CGCCCGCAAG CGCATCCACG AGGGCCC
	AMCCIACCIGGO
	GGAAAAGCC

 The DNA of claim 6, wherein one or more bases in SEQ ID NO:4 are replaced with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence in SEQ ID NO:3.

B. The DNA of claim 5, which has the base sequence in SEQ ID NO:5:

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	SEQ ID NO:5:  GCCCCTCCCT CCCCAACCG GGCCTTCCCG TGGGGGGGCC GTGACCCCTT GCGGGCCAGG 126  GCCCCTCCCT CCCCCAACCG GCCCTTCCC GCCGTGGCC TCGCCCTGC CCTGCACTAC 126  CCCCTCTTGC GCCGTGGCC TCGCCCTGCC TAAGGCGGTG 300  ACCCCTCCTGCC TCGCCCTGCC TAAGGCCGTT	0
5	TGCTCGACCT ACCCCGGGGT GCGGGCCCGG GCCTGCCTCG AGGGGGT CCTGGAGCTC	0
		20 80
		40
	LYLACTOOL COMPLY A COLOGO COLDE CHOOLINE COLDE	88
10	GGCCGGGGGG AGGTCCTGGC CTACTION GCG GTG ATC TAC GIR Leu His Val	
	CTC (AND 000 man TOT DYP 100 4 A	36
	1	
	Met Asp Pro Leu TTP 172 10 10 10 10 10 10 10 10 10 10 10 10 10	584
15	Arg Ser Phe Phe Asp Are 25 20 20 CTG AGG CGG AAG CTT CCC TAC CTG GAG GAG CTC GGG GTC AAC ACC CTG CTG AGG CGG AAG CTT CCC TAC CTG GAG GAG CTC GGG GTC AAC ACC CTG AGG CGG AAG CTT CCC TAC CTG GAG GAG GAC GAC GAT Leu Arg Arg Lys Leu Pro Tyr Leu Glu Glu Leu Gly Val Asn Thr Leu 45 Leu Arg Arg Lys Leu Pro Tyr Lou Glu Glu GAG GAC GAC GGG TAC GAT AGG AGG GAC GAC GAT ASP Gly Tyr Asp	
	CTG AGG CGG AAG CTT CCC THE Leu Glu Glu Leu G11 45	732
	CTG AGG CGG AAG CTT COO TYT Leu Glu Glu Het 45  Leu Arg Arg Lys Leu Pro Tyr Leu Glu Glu Het 45  45  40  45  45  45  45  46  47  48  48  45  47  40  48  48  48  48  48  48  48  48  48	
		780
20,	Leu Arg Arg Lys Leu FTG 40  35  TGG CTC ATG CCC TTC TTC CAG TCC CCC TTG AGG GAC GGG TAC GAS  TTP Leu Met Pro Phe Phe Gln Ser Pro Leu Arg Asp Asp Gly Tyr Asp  50  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC GAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC GAG ATC CTC CCC GTC CAC GGG ACC ATT GAG  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC  ATC TCC GAC TAC TAC CAG ATC CTC CCC GTC CAC GGG ACC CTG GAG GAC CC C	,00
	ATC TCC GAC TAC TAC CAN le Leu Pro Val ATS  Ile Ser Asp Tyr Tyr Gln lle Leu Pro Val ATS  75  Ile Ser Asp Tyr Tyr Gln lle Leu Pro Val ASP GTG ATC ATT GAG  76  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC GGG GGG ATG AAG GTG ATC ATT GAG  95  TTC ACC GTG GAC GAG GCC CAC GGC GGG GGG ATG AAG GTG ATC ATT GAG  75  TTC ACC GTG GAC GAG GCC CAC GGC GGG GGG ATG AAG GTG ATC ATT GAG  90  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC ATG AAG GTG ATC ATT GAG  90  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  90  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC ATT GAG  90	828
	The Ser Asp 170 70 CAC GGC CGG GGG ATG AAG UND THE THE Glu	
25	Ile Ser ASP TYT TY1  70  65  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC AIT GLU  95  TTC ACC GTG GAC GAG GCC CAC GGC CGG GGG ATG AAG GTG ATC AIT GLU  95  Phe Thr Val ASP Glu Ala His Gly Arg Gly Met Lys Val Ile Ile Glu  95  Phe Thr Val ASP Glu Ala His Gly Arg GO GCG CCT TGG TTC CAG GAG GCG  85	876
	Phe Thr Val ASP 85	
		924
	Leu Val Leu Asn His Thr Ser 105 TAC GTG TGG AGC GAC ACC	•
30		077
	AGG AAG CCG ASIN Ser Pro Met Arg ASP 120 TTT GAA ACC	972
	115 CGG GTC CGG GTC ATC TTC AAS Phe Glu Thi	
	AGG AAG CCG AAT AGC CGG MET ATG ASP TTP 197 125  ATG Lys Pro Asn Ser Pro Met ATG ASP TTP 197 125  ATG Lys Pro Asn Ser Pro Met ATG ASP TTP 197 125  CCG GAG AAG TAC AAG GGG GTC CGG GTC ATC TTC AAG GAC TTT GAA ACC  CCG GAG AAG TAC AAG GGC GTC CGG GTC ATG TAC TAC TGG CAC CGC  Pro Glu Lys Tyr Lys Gly Val ATG Val Ile Phe Lys ASP Phe Glu Thr  Pro Glu Lys Tyr Lys Gly Val ATG Val Ile Phe Lys ASP Phe GLU Thr  ATG CCC GTG GCC AAG GCC TAC TAC TGG CAC CGC  TAC TTT His ATG	1020
	Pro Glu Lys 191 22 135 130 130 135 GCC GTG GCC AAG GCC TAC TAC 1GG SHIS Arg	
		1068
	Pro Glu Lys Tyl Bys 135  130  TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG GCC TAC TAC TGG CAC  Ser Asn Trp Thr Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg  150  145  TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAC  TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAC  175  TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAC  175  TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAC	
	145 CAC CCC GAC 175	
	TTC TAC 166 Sin Pro Asp Let ASS 170 TTC CTG GGG GTG GAC	1116
	THE CAL GTC ATG TTO THE MAN AIR ASP LEU CAR	
	AAG GCC ATC CAC CAG Wal Met Phe Phe Trp Ala 190 CGG ACC	1164
	Phe Tyr Trp His Gin 165  AAG GCC ATC CAC CAG GTC ATG TTC TTC TGG GCC GAC CTG GGG GIG AAG  AAG GCC ATC CAC CAG GTC ATG TTC TTC TTC TGG GCC GAC CTG GGG GIG AAG  AAG GCC ATC CAC CAG GTC ATG TTC TTC TTC TAC GAG CGG GAG GGG ACC  Lys Ala Ile His Gin Val Met Phe Phe Trp Ala Asp Leu Gly Val Asp  180  TGG ATC CCC TAC CTC TAC GAG CGG GAG GGG ACC	
	AAG GCC ATC CAC CAG Val Met Phe Phe TIP ALGORITHM 190 Lys Ala Ile His Gln Val Met Phe Phe TIP ALGORITHM 190 Lys Ala Ile His Gln Val Met Phe Phe TIP ALGORITHM 190 180 GGC TTC CGC CTG GAC GCC ATC CCC TAC CTC TAC GAG CGG GAG GGG ACC	
	45	

5 .

	Gly Phe Arg Leu Asp Ala Ile	e Pro Tyr Lei	Tyr Glu Arg Glu Gly Thr 205	
	TCC TGC GAG AAC CTC CCC GAG Ser Cys Glu Asn Leu Pro Gl			212
5				260
	Lys Ala Leu Glu Glu A19 -1		235 FINE CCC CAC GGG GAC 1	308
10	Ala Asn Met Tip Flo old -	25	O THE SEC SEC ATC TTC ATG 1	356
	Gly Val His Met Ald If 192	265	270 AMC CTC AAG GAG 1	.404
15	Gly Val His Mec Ald 17  260  GCC CTA AGG CGG GAG GAC CG Ala Leu Arg Arg Glu Asp Ar			L <b>4</b> 52
	GCG GAG GGG ATC CCC GAA AC	CC GCC CAG TO	rp Ala Leu Phe Leu Arg Asn	.432
20			CG GAG GAG GAG CGG GAG TTC hr Glu Glu Glu Arg Glu Phe 315 315 315 316 317 318 319 310 310 310 310 310 310 310 310 310 310	1500
	His Asp Glu Leu III 200		315	1548
25	Met Tyr Glu Ala 191 7.20	3	30 .	1596
	Ile Arg Arg Arg Led Met 1	345	ALC CCC ACG CCC ATC GTC	1644
30	Glu Leu Leu Thr Ala Bea -	360	305	1692
30			SAC AAC CCC TTC CTC GGG GAC Asp Asn Pro Phe Leu Gly Asp 380	1740
	CGG AAC GGT GTC AGG ACC C	CCC ATG CAG T Pro Met Gln T	Trp Ser Gln Asp Arg Ile Val	1788
35	GCC TTC TCC CGC GCC CCC '	TAC CAC GCC (	Leu Phe Leu Pro Pro Val Ser	
			AAC GTG GAG GGC CAG CGG GAA ASN Val Glu Ala Gln Arg Glu 430	1836
40	Glu Gly Pro Tyl Ber 174	425	THE SEC MINE CITY GCC CITY AGG	1884
	Asn Phe His Sei Bed 200	440	445	1932
45	Asn Gln His Ala Dys 110	455	460 CAC GAG GGG GAG	1980
	Val Glu Ash Arg Arg 470		475	2028
50	CGG GTC CTG GTG GTG GCC Arg Val Leu Val Val Ala 485	AAC CTC TCC Asn Leu Ser	CGC TAC ACC CAG GCC TTT GAC Arg Tyr Thr Gln Ala Phe Asp 490	

	CTC CCC TTG GAG GCC TAC CAA GGC CTC GTC CCC GTG GAG CTC TTC TCG 2076  CTC CCC TTG GAG GCC TAC CAA GGC CTC GTC CCC GTG GAG CTC TTC TCG 2076  CTC CCC TTG GAG GCC TAC CAA GGC CTC GTC CCC GTG GAG CTC TTC TCG 2076
	CTC CCC TTG GAG GCC TAC CAA GGC CTC GTC CCC GTG GAG CTC TTC TCC Leu Pro Leu Glu Ala Tyr Gln Gly Leu Val Pro Val Glu Leu Phe Ser 505 500 CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC GTG CTC CAG CAA CCC TTC CCC CCG GTG CTC CTC CTC CTC CTC
5 -	CAG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGG CAG CAG CAG CAG CAG CAG CAG CAG CA
	CCC CAC GGC TTC GCC CTC TTC GCC CTG AAG CCC GTG GAG GCC GTG GAG GCC GCC GAG GAG GCC GCC GAG GAG GCC GCC
10	Pro His Gly Pile No. 535 530 CAC CTC CCC GAC TGG GCC GAG GAG CCC GCC CCC GAG GAG GAG CAC CTC CCC TCC CCC GAC TGG GCC GAG GAG CCC GCC CCC GAG GAG GAG His Leu Pro Ser Pro Asp Trp Ala Glu Glu Pro Ala Pro Glu Glu Ala 550 555 550 545 GAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG GTG 575 GAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG GTG 575 570 570 570 570 570 570 571 570 571 570 571 570 571 572 573
15	His Leu Pro Sel 1550 545 GAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG Val GAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC Leu Val Asp Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu Val 575 Asp Leu Pro Arg Val His Met Pro Gly GGG GAG GAG CTC CTA AAC GCC CTC 565 GAC ACC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC CTC 590 590 585
	ASP Leu PTB ATS 565  GAC ACC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC GAG AAC GCC GAA AGG GGG CGG GAG GA
20	ASP THE LEW 580  GCC CAG ACC CTG AAG GAG AAG AGC TGG CTC GCC CTC AAG CCG CAG Lys  GCC CAG ACC CTG AAG GAG AAG AGC TTP Lew Ala Lew Lys Pro Gln Lys  Ala Gln Thr Lew Lys Glw Lys Ser Trp Lew Ala Lew Lys Pro G05  Ala Gln Thr Lew Lys Glw Lys Ser TTP Lew Ala Lew Lys Pro G05  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTC CTC CTC CTC CTC CTC CTC CT
•	Ala Gln Thr Let 27 600  595  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TYC  GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TYC  600  610  610  CTC ACC CTG CTC CAG CTG GAG AAC CAC AGG ACC CTC CAG GTC TCC CTC  640  640  635
25	Val Ala Leu Leu Glo CTC GAG AAC CAC AGG ACC CTC CAG GTC ICC GGC CTC ACC CTG CTC CAG CTG GAG AAC CAC AGG ACC CTC CAG GTC ICC GAG ACC CTC ACC CTC CTC CTC CTC GAG AAC CAC AGG ACC CTC CTC GAG GTC ICC GAG ACC CTC CTC GAG AAC CAC AGG ACC CTC CTC GAG GTC ICC GAG ACC CTC CTC GCC CAG AGG CGG GAA GGC CCC GGC CTC TTC GCC CTC CTC TGG TCC CCC C
	Leu Thr Leu Bed 630 625 CCC CTC TGG TCC CCC CAG AGG CGG GAA GGC CCC GGC CTC TTC CCC CTC TGG TCC CCC CAG AGG CGG GAA GGC CCC GGC CTC TCC CCC CTC TGG TCC CCC CAG AGG CGG GAA GGC CCC GGC CCC GGC TAC TCC TCC TTG GAC CCA 645 CGC ACC CAC GGC CAG CCC GGC TAC TTC TAC GAG CTC TCC TTG GAC CCA CCC CAC GGC CAG CCC GGC TAC TTC TAC GAG CTC TCC TTG GAC CCA CCC ACC CAC GGC CAG CCC GGC TAC TTC TAC GAG CTC TCC TTG GAG GGG CCC TCC TCC TTG GAC CCA CCC TCC TCC TCC TTG GAC CCA CCC TCC TCC TTG GAC CCA CCC TCC TCC TCC TTG GAC GCG CCC TCC TCC TCC TCC TCC TTG GAC CCA CCC TCC TCC TCC TCC TCC TCC TCC TCC TCC
30	CGC ACC CAC GGC CAG CCC GGC TAC TTC TAC GAG CTC TCC TTG GAG Pro  CGC ACC CAC GGC CAG CCC GGC TAC TTC TAC GAG CTC TCC TTG GAG  Arg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro  665  665  666  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGG  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGG  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGG  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGC  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGC  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGC  GGC TTC TAC CGC CTC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGC  GGC TTC TAC CGC CTC CTC CTC CTC GCC CTC CTC CTC GCC CTC CT
	GGC TTC TAC CGC CTC CTC GCC CGC CTT AAG GAG GGG TTT GAG GGG GTC GGC CTT AAG GAG GGG TTT GAG GGG GGC GGC GGC CTT AAG GAG GGG TTT GAG GGG GGC GGC GGC GGC GGC GGC GGC GGC
35	Gly Phe Tyl Arg
40	Arg Ser Leu Arg 695 690 GAG GCC GTG GAC CTC CTC CGG CCG GGA CTC GCG GGG GAG GGG GAG GCC GTG GAC CTC CTC CGG CCG GGA CTC GCG GGG GAG GGG GLU Ala Val Asp Leu Leu Arg Pro Gly Leu Ala Ala Gly Glu Gly 720 715 705 TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG GAG CTC GTC GAC GGG GTC CTG GAC CGC ACG GAG CTC GTC GAC GGG GTC CTG GAC CGC ACG GAG CTC GTC GAC GGG GTC CTG GAC CGC ACG GAG CTC GTC GAC GGG GTC CTG GAC CGC ACG GAG CTC GTC GTC GTC GTC GTC GTC GTC GTC GT
40	Glu Ala Val Asp 710 705 TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG TGG GTC CAG CTC GGC CTC GTC CAA GAC GGG GGC CTG GAC CGC ACG TTP Val Gln Leu Gly Leu Val Gln Asp Gly Gly Leu Asp Arg Thr Glu 735 730 735 CGG GTC CTC CCC CGC CTG GAC CTC CCC TGG GTT CTC CGG CCC GAA GGG CGG GTC CTC CCC CGC CTG GAC CTC CCC TGG GTT CTC CGG CCC GAA GGG 750 750 750 745
4	CGG GTC CTC CCC CGC CTG GAC CTC CCC TGG GTT CTC CGG CCC GAL Gly  CGG GTC CTC CCC CGC CTG GAC CTC CCC TGG GTT CTC CGG CCC GAL Gly  Arg Val Leu Pro Arg Leu Asp Leu Pro Trp Val Leu Arg Pro Glu Gly  750  740  GGC CTC TTC TGG GAG CGG GGC GCC TCC AGA AGG GTC CTC GCC CTC ACG  GGC CTC TTC TGG GAG CGG GGC GCC TCC AGA AGG GTC CTC GCC CTC ACG  765  765  765  767  768  769  769  760  760  760  760  760  760
	GGC CTC TTC TGG GAG CGG GGC GCC TCC AGA AGG GTC CTC GCC CTC TTC TGG GAG CGG GGC AGA AGG GTC CTC GCC CTC TTC TGG GAG AGG CTC TTC GCC GCC CTG GAG 765 760 765 765 760 765 765 760 765 765 760 765 760 765 765 760 765 760 765 760 765 765 760 765 765 760 760 760 760 760 760 760 760 760 760
	Gly Leu Phe 119 760 760 760 760 760 775 7760 GGC CCC CGG GGC CCC CAG GAC CTC TTC GCC GGC CTC GGC GGC GGC GGC CTC GGG GGC CTC GGC GG
	Gly Ser Leu Flo 1775 770 GTC CGG CTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CGG CTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GTC CTG GAA AGC CTT CCC CGC CTC CTG GGG CAC GCC GCC CTC CTG CTG GAA AGC CTT CCC CGC CTC CTG GGG CAC GCC CTC CTG GAA AGC CTT CCC CGC CTC CTG GAA AGC CTT CCC CGC CTC CTG GAA AGC CTT CCC CTG CTG GAA AGC CTT CTG GAA AGC CTT CTG GAA AGC CTT CTG GAA AGC CTT CCC CTG CTG GAA AGC CTG GAA A

				800	2988	
			705 656	CTG GTC	2900	
			CAG ACC GAA GCC	a Leu Val		
	790	) - CCC CTG CAC	Clu Thr Glu Al	B15	3036	
_	- com CCC	GGG GOO Leu His	GIG -	T GGG GAG	3000	
785	CA GGC CTC CIT Pr	O GIY ALC BIO	CAC CGG GCC CT	Gly Glu		
ACC C	790 CA GGC CTC CTT CCC Pro Gly Leu Leu Pr 805	- CCC CTC CTC	Wie Arg Ala Le	30 0-2	3084	
Thr b	790 CA GGC CTC CTT CCC ORO Gly Leu Leu Pr 805	C CTC Goo Leu Leu	) HIS - B.	CC CTC GGG	300	
_	CA GGC CTC CTT CCC CTC GGY Leu Leu Pr CTC CTC GGG GTG CG Leu Leu Gly Val Al GAG GGG GTG GTG GGU GIY Val CGU GIY CAG CTG CG	cg Leu Ale 825	GC CGC GG	Leu Gly		
_ ccc	Leu Gly Var	CAC CCC CTG	TOU GLY ATG	111 - 	3132	
Arg	820 GTG G	GG GGC Cho Pro Le	iu 160 845	THE GGC GCG	320	
	Leu Leu GIY  820  GAG GGG GTG GTG  Glu Gly Val Val  835  TTC CTG GAG CTG  Phe Leu Glu Leu	ly Gly 840	G CTC GTG GCC	Leu Gly Ala	3	
GTG	Clu Gly Val Val	GAG GTG TA	Tell Val Ala	1000	3180	
Val	835 CTG	SAG GGG GAL Val T	At 160 860	GCC TAC GAG	J	
	MTC CTG GAO	Glu Gi	-c GCC CGC CIO	Ala Tyr no	, D	
GCC	phe Leu Glu	BSS GAG GAC C	Ala Arg Leu	7 86	3228	3
Als	GAG GGG GTG GTG GIU GIY Val Val GIU S35 C TTC CTG GAG CTG GAG CTG GAG Leu Glu Leu S50	GTG GAU Glu ASP	875 - CAG	GCG GAG CT		
	AAG CGG GOO Thr	Val Gra	and GCC CTG Gill	. Ala Glu D	<b>-</b>	
GA	Glu Gly Val 835 TTC CTG GAG CTG Phe Leu Glu Leu 850 A AAG CGG GGC ACG A AAG CGG GGC ACG A Lys Arg Gly Thr	870 GCC CTC	Clu Ala Leu Gio	895	mc 327	6
: G1	Phe Leu Glu Bob B50 A AAG CGG GGC ACG A AAG CGG GGC Thr GG GAG CGG GCC GTG Ala Glu Arg Ala Val AGG GCC TTT GCC GAG AGG GCC TTT	CAC CIO Ala Leu	990 - 666	GCC TTC	10	
C.	rg GAG CGG GOO Val	His Dec	CAC CTC CAC GCC	a Ala Phe	760	
37	al Glu Arg Are 885	GOTG GCC GAC	uis Leu His AI	910	age 337	24
V	-m GCC GAG	GAG Wal Ala ASP	nic GA	G GAG GCG	C) A	
п	GG GCC TTT Ala G1	a GIA 40- 905	GAG GCC CTG	lu Glu Ala	0-1	
20	TED Ala Phe 400	CTC CCC GAG	Glu Ala Leu 9	25	GAG 33	372
•	al Glu Arg Ala 885 GG GCC TTT GCC GAG TTP Ala Phe Ala Gl CAA GCC TAC CGC TC Gln Ala Tyr Arg Sc Gln Ala Tyr Arg Sc Gln Ala Tyr Arg Sc	C GCC Leu Pro GI	ב האר C'	TC CAC CGG	Glu	
	CAA GCC TAG AIG SE	920 CC	G GCG GAG THE I	eu His Arg		420
	Gln Ala 192	-C CCC GAG GTG AL	a Ala Glu Min	C A&G	GCC 3	420
	CAA GCC TAC CGC TCG Ala Tyr Arg StG ACG CGG CAC ATT TYR ARG HIS ME	TG GCC GAG GTG GCC let Ala Glu Val Al 935 CGC AAG CGC ATC CA Arg Lys Arg Ile H	TGG TGG	AG GCC AND	, Ala	c
25	TGG ACG AIG HIS N	935	AC GAG CGC TIP	Gln Ald 2	960 ,	3429
	Trp Till	CC AAG CGC ATO H	is Glu Alg		•	J ==
	GAA AGG Pro Ala	950		•	- acmC	3489
	Glu Kra			TOTAL AGA	CGGCC10	3549
30	77"		miles	GGG ~C(	GGCAGA.	3600
	GGA AAA Ala Gly Lys Ala	CCCGGGC	CACGGGGGACG GCG	TCCCTAA G		
	963	CCCTTC AGCCCCGGCC GGCGCT TCTTGGCCCG	GCGGTAGG CAC	JICCC 11-		
	TAGGCGCCCG GTAG	GGCGCT TCTTAGCC	GUACCOO			
•	CTCGGGGAGG AGGC	CCGTGG TGGGG	_	Thormus.		
35	GGCGCACACC GCCC	CCCTTC AGCCCCGGGC GGCGCT TCTTGGCCCG TGGGGTAGCC	arganism of the genus	Home	- the enzyme	e of clai
	500	iar	MOULANIE		- the BULLY""	-

- 9. The DNA of claim 5, which is derived from a microorganism of the genus Thermus.
- 10. A replicable recombinant DNA which contains a self-replicable vector and a DNA encoding the enzyme of claim 1. 40
  - 11. The replicable recombinant DNA of claim 10, wherein said DNA contains a base sequence selected from the group The replicable recombinant DINA of claim 10, wherein said DINA contains a base sequence selected from the group consisting of the base sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary
  - 12. The replicable recombinant DNA of claim 11, wherein said DNA is obtained by replacing one or more bases in SEQ The replicable recombinant DINA of claim 11, wherein said DNA is obtained by replacing one or more bases in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence is SEQ ID NO:3 45
    - 13. The replicable recombinant DNA of claim 10, wherein said DNA has the base sequence in SEQ ID NO:5.
    - 14. The replicable recombinant DNA of claim 10, wherein said DNA is derived from a microorganism of the genus
      - 15. The replicable recombinant DNA of claim 10, wherein said self-replicable vector is plasmid vector Bluescript II SK
      - 16. A transformant which is prepared by introducing into an appropriate host a replicable recombinant DNA which con-

- tains a DNA encoding the enzyme of claim 1 and a self-replicable vector. 17. The transformant of claim 16, wherein said DNA has a base sequence selected from the group consisting of the the manifestrum of claim 10, whilefull said DIAC has a base sequences thereunto, and complementary base sequences to base sequence in SEQ ID NO:4, homologous base sequences thereunto.
- 18. The transformant of claim 17, wherein the said DNA is obtained by replacing one or more bases in the base sequence in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid
- 19. The transformant of claim 16, wherein said DNA has the base sequence in SEQ ID NO:5.
- 20. The transformant of claim 16, wherein said DNA is derived from a microorganism of the genus Thermus.
- 21. The transformant of claim 16, wherein said self-replicable vector is plasmid vector Bluescript II SK(+) or pKK223-3.
  - 22. The transformant of claim 16, wherein said host is a microorganism of the species Escherichia coli.
  - 23. A process for preparing a recombinant enzyme, which comprises:

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- culturing a transformant, prepared by introducing into an appropriate host a recombinant DNA containing both a self-replicable vector and a DNA encoding the recombinant enzyme of claim 1, in a nutrient culture medium
- 24. The process of claim 23, wherein said DNA has a base sequence selected from the group consisting of the base collecting the formed enzyme from the resultant culture. sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary base sequences to these
- 25. The process of claim 24, wherein the said DNA is obtained by replacing one or more bases in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence in SEQ ID 30
  - 26. The process of claim 23, wherein said DNA has the base sequence in SEQ ID NO:5.
  - 27. The process of claim 23, wherein said DNA is derived from a microorganism of the genus Thermus.
  - 28. The process of claim 23, wherein said self-replicable vector is plasmid vector Bluescript II SK(+) or pKK223-3.
  - 29. The process of claim 23, wherein said host is a microorganism of the species Escherichia coli. 40
    - 30. The process of claim 23, wherein the recombinant enzyme formed in the nutrient culture medium is recovered by centrifugation, filtration, concentration, sating out, dialysis, separatory sedimentation, ion-exchange chromatography, gel filtration chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and/or
    - 31. An enzymatic conversion method of maltose, which comprises a step of allowing the recombinant enzyme of claim
    - 32. The method of claim 31, wherein the step comprises coexisting an effective amount of the recombinant enzyme in an aqueous medium containing maltose up to 50 w/w %, and subjecting the resultant mixture to an enzymatic 50
    - 33. The method of claim 31, wherein the resulting reaction mixture contains at least about 50 w/w % trehalose, on a dry solid basis. 55

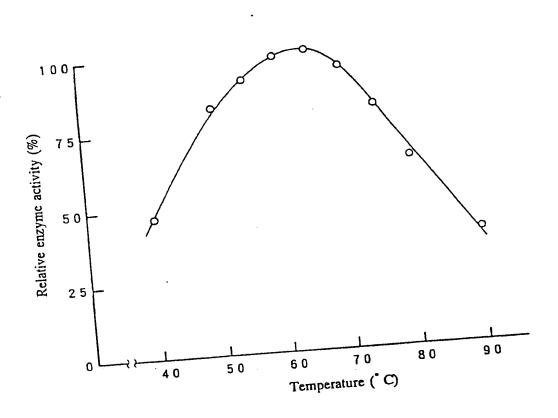


FIG.1

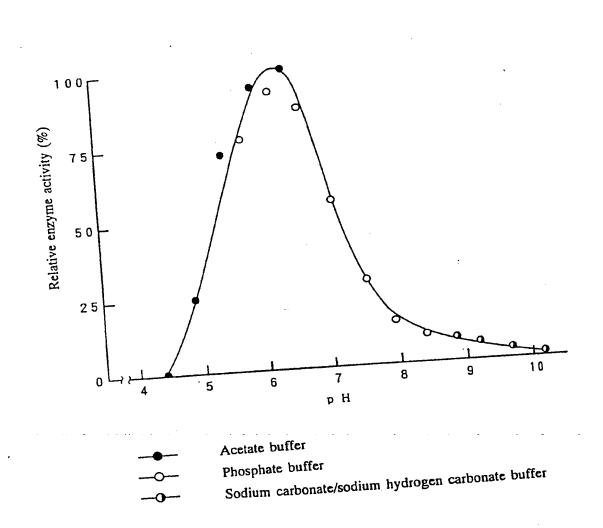


FIG.2

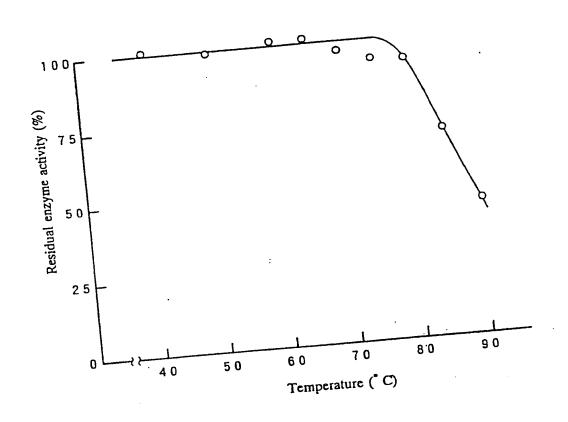


FIG.3

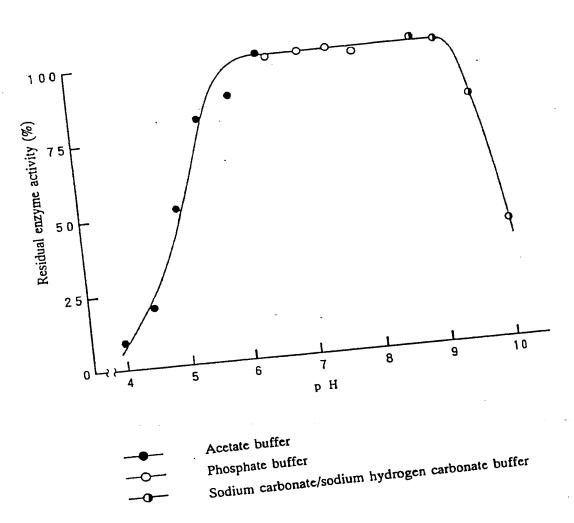


FIG.4

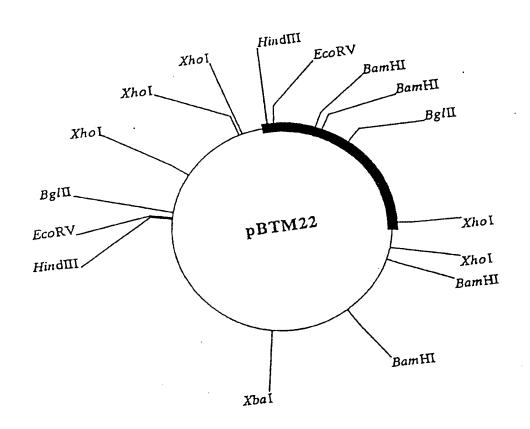


FIG.5